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(21) International Application Number: PCT/US99/17578 (22) International Filing Date: 4 August 1999 (04.08.99) (30) Priority Data: 60/095,269 4 August 1998 (04.08.98) US 60/099,973 11 September 1998 (11.09.98) US 60/118,783 5 February 1999 (05.02.99) US (71) Applicant (for all designated States except US): IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BIRD, Timothy, A. [GB/US]; 10804 Olallie Lane, N.E., Bainbridge Island, WA 98110 (US). VIRCA, G., Duke [US/US]; 16690 S.E. 50th Place, Bellevue, WA 98006 (US). (74) Agents: GARRETT, Arthur, S. et al.; Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: IKR-1 AND IKR-2, PROTEIN KINASES WHICH ARE RELATED TO THE I KAPPA B KINASES			
(57) Abstract <p>The invention is directed to purified and isolated kappa B kinase-related kinases 1 and 2 (IKR-1 and IKR-2) polypeptides and fragments thereof, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and the uses of the above.</p>			

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IKR-1 AND IKR-2, PROTEIN KINASES WHICH ARE RELATED TO THE I KAPPA B KINASES

CROSS-REFERENCE TO RELATED APPLICATION

The present invention is based on U. S. provisional application number 60/118,783, filed February 5, 1999, which is based on U.S. provisional application number 60/099,973, filed September 11, 1998, and U. S. provisional application 60/095,269, filed August 4, 1998. Each of these applications is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to purified and isolated novel I kappa B kinase-related kinases 1 and 2 (IKR-1 and IKR-2) polypeptides and fragments thereof, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and uses thereof.

Description of Related Art

The transcription factor NF- κ B (nuclear factor κ B) is composed of homo- or heterodimers of proteins from the Rel family of transcription factors. The major genes regulated by NF- κ B are immune, viral, and inflammatory response genes (C.H. Regnier et al., *Cell*, Vol. 90: 373-383, July 1997). When NF- κ B is not involved in active transcription of these genes, it is located in the cytoplasm bound to the inhibitory protein I κ B which regulates the activity of NF- κ B (J.A. DiDonato et al., *Nature*, Vol. 388: 548-554, August 1997). I κ B, when bound to NF- κ B molecules, masks the nuclear localization signal of NF- κ B, thus inactivating the protein.

In response to extracellular cytokines or other pro-inflammatory stimuli, I κ B molecules are quickly phosphorylated by intracellular kinases such as I Kappa B kinases α and β (IKK α and IKK β). Once activated, IKK α and IKK β specifically phosphorylate I κ B at specific serine residues in the N-terminus or I κ B alpha at serine residues 32 and 36, thereby targeting this molecule for proteolytic destruction. The outcome of I κ B phosphorylation and destruction is the release and subsequent translocation of NF- κ B from the cytosol to the nucleus where it engages transcriptional regulatory sites on a number of immune related and pro-inflammatory genes.

The eukaryotic protein kinases make up a large and rapidly expanding family of proteins related on the basis of homologous catalytic domains. Spurred by the development of gene cloning and sequencing methodologies, distinct protein kinase genes have been identified from a wide selection of invertebrates and lower eukaryotes, including *Drosophila*, *Caenorhabditis elegans*, *Aplysia*, *Hydra*, *Dictyostelium*, and budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeast. Homologous genes have also been identified in higher plants. Protein kinases, however, are not limited to the eukaryotes. Enzyme activities have been well documented in prokaryotes, but the prokaryotic protein kinase genes are not obviously homologous to those of the eukaryotes.

Given the important function of kinases in general and IKK's specifically, there is a need in the art for additional members of the kinase family. In addition, in view of the continuing interest in protein research, the discovery, identification, and roles of new proteins, such as protein kinases, are at the forefront of modern molecular biology and biochemistry. Despite the growing body of knowledge, there is still a need in the art for the identity and function of proteins having kinase activities. In addition, because there is an unmet need for therapeutic

compounds which interfere with activation of NF- κ B and because protein kinases are useful biochemical reagents, there is also need in the art for the continued discovery of unique members of the IKB protein kinase family and potential therapeutic targets thereof.

In another aspect, the identification of the primary structure, or sequence, of an unknown protein is the culmination of an arduous process of experimentation. In order to identify an unknown protein, the investigator can rely upon a comparison of the unknown protein to known peptides using a variety of techniques known to those skilled in the art. For instance, proteins are routinely analyzed using techniques such as electrophoresis, sedimentation, chromatography, sequencing and mass spectrometry.

In particular, comparison of an unknown protein to polypeptides of known molecular weight allows a determination of the apparent molecular weight of the unknown protein (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)). Protein molecular weight standards are commercially available to assist in the estimation of molecular weights of unknown protein (New England Biolabs Inc. Catalog:130-131, 1995; J. L. Hartley, U.S. Patent No. 5,449,758). However, the molecular weight standards may not correspond closely enough in size to the unknown protein to allow an accurate estimation of apparent molecular weight. The difficulty in estimation of molecular weight is compounded in the case of proteins that are subjected to fragmentation by chemical or enzymatic means, modified by post-translational modification or processing, and/or associated with other proteins in non-covalent complexes.

In addition, the unique nature of the composition of a protein with regard to its specific amino acid constituents results in unique positioning of cleavage sites within the protein. Specific fragmentation of a protein by chemical or enzymatic cleavage results in a unique "peptide fingerprint" (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977; M. Brown et al., *J. Gen. Virol.* 50:309-316, 1980). Consequently, cleavage at specific sites results in reproducible fragmentation of a given protein into peptides of precise molecular weights. Furthermore, these peptides possess unique charge characteristics that determine the isoelectric pH of the peptide. These unique characteristics can be exploited using a variety of electrophoretic and other techniques (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)).

Fragmentation of proteins is further employed for amino acid composition analysis and protein sequencing (P. Matsudaira, *J. Biol. Chem.* 262:10035-10038, 1987; C. Eckerskorn et al., *Electrophoresis* 1988, 9:830-838, 1988), particularly the production of fragments from proteins with a "blocked" N-terminus. In addition, fragmented proteins can be used for immunization, for affinity selection (R. A. Brown, U.S. Patent No. 5,151,412), for determination of modification sites (e.g. phosphorylation), for generation of active biological compounds (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 300-301 (Prentice Hall, 6d ed. 1991)), and for differentiation of homologous proteins (M. Brown et al., *J. Gen. Virol.* 50:309-316, 1980).

In addition, when a peptide fingerprint of an unknown protein is obtained, it can be compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015, 1993; D. Fenyo et al., *Electrophoresis* 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet

site: prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearchForm.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare these molecular weights to protein molecular weight information stored in databases to assist in determining the identity of the unknown protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in determining the number of fragmented peptides and their molecular weight should result in enhanced likelihood of success in the identification of unknown proteins.

In addition, peptide digests of unknown proteins can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec.* 5:976-989 (1994); M. Mann and M. Wilm, *Anal. Chem.* 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec.* 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site: www.lsbcc.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search and ProFound programs described above. Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using tandem mass spectrometry.

Thus, there also exists a need in the art for polypeptides suitable for use in peptide fragmentation studies, for use in molecular weight measurements, and for use in protein sequencing using tandem mass spectrometry.

SUMMARY OF THE INVENTION

The invention aids in fulfilling these various needs in the art by providing isolated IKR-1 and IKR-2 nucleic acids and polypeptides encoded by these nucleic acids. Particular embodiments of the invention are directed to isolated IKR nucleic acid molecules (IKR-1 and IKR-2) comprising the DNA sequence of SEQ ID NOs:1 and 3 and isolated IKR nucleic acid molecules encoding the amino acid sequences of SEQ ID NOs:2 and 4, as well as nucleic acid molecules complementary to these sequences. The invention also encompasses recombinant vectors that direct the expression of the nucleic acid molecules of the invention and host cells stably or transiently transformed or transfected with these vectors.

In addition, the invention encompasses methods of using the nucleic acids noted above to identify nucleic acids encoding proteins having kinase function and to study cell signal transduction and activation of transcription factors such as the NF- κ B family of molecules.

The invention also encompasses isolated polypeptides and fragments thereof encoded by these nucleic acid molecules. The invention further encompasses methods for the production of these polypeptides, including culturing a host cell under conditions promoting expression and recovering the polypeptide from the culture medium. Especially, the expression of these polypeptides in bacteria, yeast, plant, insect, and animal cells is encompassed by the invention.

In general, the polypeptides of the invention can be used to study cellular processes such as immune regulation, cell proliferation, cell death, cell migration, cell-to-cell interaction, inflammatory responses, and cell signal transduction. In addition, these polypeptides can be used to identify proteins associated with IKR kinases.

In addition, the invention includes assays utilizing these polypeptides to screen for potential inhibitors of their kinase activity and methods of using these polypeptides as therapeutic agents for the treatment of diseases mediated by IKR polypeptide counter-structure molecules (including substrates, regulatory proteins, small molecules, etc.). Further, methods of using these polypeptides in the design of inhibitors thereof are also an aspect of the invention.

The invention further provides a method for using these polypeptides as molecular weight markers that allow the estimation of the molecular weight of a protein or a fragmented protein, as well as a method for the visualization of the molecular weight markers of the invention thereof using electrophoresis. The invention further encompasses methods for using the polypeptides of the invention as markers for determining the isoelectric point of an unknown protein, as well as controls for establishing the extent of fragmentation of a protein .

Further encompassed by this invention are kits to aid in these determinations.

Further encompassed by this invention is the use of the IKR nucleic acid sequences, predicted amino acid sequences of the polypeptide or fragments thereof, or a combination of the predicted amino acid sequences of the polypeptide and fragments thereof for use in searching an electronic database to aid in the identification of sample nucleic acids and/or proteins.

Isolated polyclonal or monoclonal antibodies that bind to these polypeptides are also encompassed by the invention, as well as the use of these antibodies to aid in purifying IKR polypeptides.

BRIEF DESCRIPTION OF THE FIGURES

This invention will be more fully described with reference to the drawings in which:

Figure 1 is the nucleotide sequence of IKR-1 SEQ ID NO:1;

Figure 2 is the nucleotide sequence of IKR-2 SEQ ID NO:3;

Figure 3 is the amino acid sequence of IKR-1 SEQ ID NO:2; and

Figure 4 is the amino acid sequence of IKR-2 SEQ ID NO:4.

DETAILED DESCRIPTION OF THE INVENTION

The nucleic acid molecules encompassed in the invention include the following nucleotide sequences:

NAME:IKR-1

Nucleotide sequence:

1	CACAGGAAAC	AGCTATGACC	ATGATTACGC	CAAGCTCGAA	ATTAACCCTC
51	ACTAAAGGGA	ACAAAAGCTG	GAGCTCCACC	GCGGTGGCGG	CCGCTCTAGA
101	ACTAGTGGAT	CCCCCGGGCT	GCAGGAATTC	CGGCCTGGGA	CTGGGTACCC
151	CACTGCTCTC	AGAGAGGCAG	GAAAGAGACC	TTCAGGCTCA	AGACCATCAC
201	CTGCTTTGCC	TGTGGATCCT	GGGGGGCCCC	ATAGCTACCA	GGATCTTCTA
251	GGCACTGCCC	AGGATTGACT	TCAAGGCCTG	AATCCCTGGG	GGTGCCACCC
301	AGTTCCACAA	GTCTGCATTG	CCCTGCAACT	GAGATAGGAG	ATGGGGAAGA
351	AGATAGCCAA	GCCCAGGAGA	TGCAGAGTAC	CACTAACTAC	CTGTGGCATA
401	CTGATGACCT	GCTAGGGCAG	GGGGCCACTG	CCAGTGTGTA	CAAGGCCCGA
451	AACAAGAAAT	CCGGGGAGGT	GGTTGCTGTA	AAGGTCTTCA	ACTCAGCCAG
501	CTATCGGCGA	CCTCCTGAGG	TTCAGGTGAG	GGAGTTTGAG	GTCCTGCGGA
551	GGCTGAATCA	CCAGAACATC	GTGAAGCTAT	TCGCAGTGGA	GGAAACGGGA

601	GGCAGCCGGC	AGAAGGTGCT	AATCATGGAG	TACTGCTCCA	GTGGGAGCCT
651	GCTGAGCGTG	CTGGAAGACC	CTGAGAACAC	GTTCCGGGCTT	TCTGAAGAGG
701	AGTTCCTAGT	GGTGCTGCGC	TGTGTGGTGG	CTGGCATGAA	CCACCTGCGG
751	GAGAATGGCA	TTGTCCATCG	GGACATCAAA	CCTGGGAACA	TCATGCGCCT
801	GGTGGGCGAG	GAGGGGCAGA	GCATCTATAA	GCTGTCTGAC	TTCGGGGGCTG
851	CCCGCAAGCT	GGACGATGAT	GAGAAGTTTG	TTTCTGTCTA	TGGTACAGAG
901	GAATACTGTC	ACCCTGACAT	GTATGAGCGT	GCAGTGCTGC	GCAAACCCCA
951	GCAAAAGGCA	TTTGGTGTGA	CTGTGGATCT	CTGGAGTATT	GGGGTGACCC
1001	TGTACCACGC	AGCCACAGGC	AGTCTGCCCT	TCATCCCCTT	CGGTGGGCCC
1051	CGGCGCAACA	AAGAGATCAT	GTACAGAATC	ACCACAGAGA	AGCCAGCCGG
1101	GGCCATTTCA	GGGACTCAGA	AGCACGAAAA	TGGTCCCTTG	GAGTGGAGCT
1151	ACAGCCTCCC	CATCACCTGT	AGACTGTCCA	TGGGACTGCA	GAACCAGCTG
1201	GTGCCCATCC	TGGCCAACAT	CCTGGAGGTG	GAAGAGGATA	AGTGCTGGGG
1251	CTTTGATCAG	TTCTTCGCGG	AGACCAGTGA	CATTCTGCAG	CGAACGGTCA
1301	TCCACGTCTT	TTCCCTACCC	CAGGCCGTTT	TGCATCATGT	CTACATCCAC
1351	GCCCACAACA	CGATTGCCAT	CTTTTTGGAG	GCTGTATATG	AGCAGACCAA
1401	CGTGACCCCC	AAACACCAGG	AGTACCTCTT	CGAGGGTCAC	CCTTGTGTCC
1451	TTGAGCCAAG	CCTCTCAGCC	CAGCACATCG	CCCACACAGC	TGCCAGCAGC
1501	CCTCTAACTC	TGTTCAGCAT	GTCCAGCGAC	ACACCTAAGG	GGCTGGCCTT
1551	CAGGGACCCT	GCTCTGGATG	TCCCAAAGTT	CGTCCCTAAG	GTTGACCTAC
1601	AGGCCGATTA	CAGCACAGCT	AAGGGGGTGC	TGGGCGCTGG	CTACCAGGCC
1651	CTGTGGCTGG	CGCGGGTCCT	GCTGGATGGA	CAGGCGTTGA	TGCTTCGGGG
1701	GTTACATTGG	GTCCTGGAGG	TGCTTCAGGA	CACGTGCCAG	CAGACACTGG
1751	AGGTCACACG	GACAGCCCTC	CTCTACCTCA	GCAGCAGCCT	GGGCACTGAA
1801	AGGTTTCAGCA	GTGGAGCGGG	GATGCCTGAC	GTCCAGGAAC	GAAAGGAGGC
1851	CACAGAGCTA	AGAACCAGGC	TGCAGACTCT	CTCAGAGATC	CTGTCTAAAT
1901	GTTCCCACAA	TGTCACAGAA	ACCCAAAGGA	GCCTGAGCTG	TCTGGGTGAA
1951	GAGCTTTTAA	AGAACCAGGA	CCAGATTTCAT	GAGGATAACA	AAAGTATCCA
2001	GAAGATTTCAG	TGTTGTTTGG	ACAAGATGCA	CTTCATCTAC	AAACAGTTCA
2051	AGAAATCCAG	GATGAGGCCA	GGGCTCAGCT	ACAATGAGGA	GCAGATCCAC
2101	AAGCTGGATA	AGGTAAATTT	CAGTCATCTA	GCCAAGAGGC	TGCTGCAGGT
2151	GTTCCAGGAG	GAGTGTGTGC	AGACGTATCA	GGTGTGCTG	GTCACACACG
2201	GCAAGCGGAT	GAGGCAGGTG	CAGAGGGCCC	AGAACCACCT	GCATCTCATT
2251	GGCCACTCTG	TGGCCACCTG	TAACTCGGAA	GCCCAGGAGG	CCCAGGAGAG
2301	TCTGAACAAG	ATCTTTGATC	AGCTCCTTCT	GGACAGAGCT	TCCGAACAGG
2351	GAGCTGAGGT	GTCACCGCAA	CCTATGGCTC	CTCATCCCGG	CCCTGATCCG
2401	AAGGACCTGG	TCTTCCACAT	GCAGGAGCTT	TGTAATGATA	TGAAGCTATT
2451	GGCCTTTGAT	CTCCAGGACA	ACAACCGACT	CATCGAACGG	TTACATAGAG
2501	TTCCATCGGC	ACCAGATGTC	TGAGCTCCCT	GGGGGTTTAC	AAGGCACTCA
2551	GAAGCAATAG	AAACATTTCAT	ATTGTACCCC	TACACTGTGA	GACCAAATTC
2601	AGGGCAAGTT	CTGGTTCCAT	CTCACTAGCC	TACCTCCCTC	TTGGCCATTG
2651	GCCATTGGCC	AACAAACTAG	CATTACTTTG	ACTGTCCTCT	TGGGAAGCAG

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2701 CTAGGACAGG GACTCCTGGC CATCCCAGGC AGTATCTACA GAAGAGACCA
2751 TGCGGCTACC ACAGCCTTAT CAAGACACCA AGACTGTTCT TCCTCACCCA
2801 GGCTCTGGAG GTCTGGTCTT GGAAAGAAAA GGCTCAGCCC TCTCAGCTC
2851 TGCACTTCCC AGGACCAGCA GGCGTCTCCT GTGGCTTCTC CTGCCTCTCC
2901 AGGGTGCTGG ATCAGAATGC TTATTCTTGG TTGTTTCCTG TGCTGCTTCC
2951 TGAGTGTCCC CATCCCTGGC CTCAGGCAAC CCACAAACGG CCCCTCTGTG
3001 CTTGGTCTAG ATGCACCTGC ATTTGAGAAA GTGGGTGGTT GAGGCTAACT
3051 GCTGGTGCTT TGAGGATTCT CCTTGACCTT TTCTCCGAGG AACGCTTGGT
3101 TCTAAGAAAC AGCTGGTCAG TATCAACCAC AGCCATGCTA ACTGGACAGA
3151 TGTTGGAACC CAAAGTCTTA AGGAGAGAGC AGGCCTGCAC CTTCAGACAT
3201 GGAATAAATA CATCGCCTTT TCTGTTTAAA AAAAAAAAAA AAAAACCGGA
3251 ATTCGATATC AAGCTTATCG ATACCGTCGA CCTCGAGGGG GGGCCCGGTA
3301 CCCAATTCGC CCTATAGTGA GTCGTATTAC AATTCACTGG CCGTCGTTTT
3351 ACAACGTCGT GACTGGGAAA ACCCTGGCGT TACCC (SEQ ID NO:1)

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NAME: IKR-2

Nucleotide sequence:

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1 TTGGGTAACG CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGCCA
51 GTGAATTGTA ATACGACTCA CTATAGGGCG AATTGGGTAC CGGGCCCCCC
101 CTCGAGGTCG ACGGTATCGA TAAGCTTGAT ATCGAATTCC GGCCTCGCG
151 GGCATACATG CAAATCTCTT CTCCCCCTT ATCGTGAGGA GAAGCGCCTG
201 GACAAGCCGA GATGCAGAGC ACCTCCAACC ATCTGTGGCT CCTGTCTGAT
251 ATCCTAGGCC AGGGGGCCAC TGCAAATGTC TTCCGAGGAA GGCATAAGAA
301 AACTGGTGAT CTCTATGCTG TCAAAGTATT TAATAACATA AGCTTCCTTC
351 GCCCAGTGGA TGTTCAAATG AGAGAATTTG AAGTGTTAAA AAAACTCAAT
401 CACAAAAACA TTGTCAAGTT ATTTGCTATT GAAGAGGAGA CAACAACAAG
451 ACATAAAGTG CTTATTATGG AGTTTTGTCC CTGTGGGAGT TTATACTG
501 TTCTAGAGGA GCCGTCCAAT GCGTATGGAC TTCCAGAATC AGAATTTCTC
551 ATTGTCTTAC GAGATGTGGT GGGCGGGATG AATCATCTCC GAGAGAACGG
601 CATAGTGCAC CGAGATATCA AGCCAGGCAA CATCATGCGC GTCATAGGGG
651 AGGACGGCCA GTCTGTGTAC AAACTCACGG ATTTCCGGCG CGCTCGAGAG
701 CTGGAGGACG ATGAGCAGTT TGTGTCTCTG TACGGCACAG AAGAGTACCT
751 GCATCCGGAC ATGTATGAAA GGGCAGTGCT AAGAAAGGAC CATCAGAAGA
801 AGTACGGGGC TACCGTTGAT CTGTGGAGTG TTGGAGTGAC ATTCTACCAT
851 GCAGCCACGG GGTGCTGCC GTTTAGACCC TTCGAGGGGC CTCGGAGGAA
901 CAAAGAAGTA ATGTATAAAA TAATCACTGG GAAGCCGTCT GGTGCAATAT
951 CTGGAGTACA GAAAGCAGAA AACGGACCAA TTGACTGGAG TGGAGACATG
1001 CCTCTCTCCT GTAGTCTTTC TCAGGGTCTT CAGGCACTGC TTACCCCACT
1051 TCTTGCAAAC ATACTTGAAG CTGATCAGGA GAAGTGCTGG GGTTTTGACC
1101 AGTTCTTTGC AGAGACCAGT GATGTGCTTC ACCGAATGGT GATCCATGTC

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1151 TTCTCGCTAC AACACATGAC GGCGCATAAG ATTTACATTC ACAGCTATAA
1201 CACTGCTGCT GTGTTCCATG AACTGGTCTA TAAACAAACC AAGATTGTTT
1251 CCTCAAATCA AGAACTTATC TACGAAGGAC GACGCTTAGT CCTAGAACTC
1301 GGACGACTAG CCCAGCATTT TCCTAAAACC ACAGAGGAAA ATCCTATCTT
1351 TGTCACGAGC CGGGAACAAC TCAATACCGT AGGACTGAGA TATGAAAAAA
1401 TTTCCCTCCC TAAAATACAT CCACGCTATG ATCTGGATGG GGACGCCAGC
1451 ATGGCCAAGG CAGTGACGGG GGTTGTGTGC TACGCCTGCA GAACTGCCAG
1501 TACCCTGCTG CTCTATCAAG AATTAATGCG AAAGGGGGTA CGGTGGCTGG
1551 TTGAACTGGT TAAGGATGAT TACAACGAGA CCGTCCACAA GAAGACGGAG
1601 GTAGTGATCA CACTGGATTT CTGCATCAGG AACATTGAGA AGACTGTGAA
1651 AGTGTATGAG AAGTTGATGA AGGTCAACCT GGAAGCCGCA GAGCTGGGTG
1701 AGATTTTCTAGA CATAACACACC AAGCTGCTGA GACTTTCCAG TTCTCAGGGA
1751 ACAATAGAAA GCAGTCTTCA GGACATCAGC AGCAGGCTGT CTCCAGGGGG
1801 CTTGCTGGCC GACACCTGGG CACATCAAGA AGGCACGCAT CCAAGAGACA
1851 GGAATGTAGA AAAACTGCAG GTCCTGTTGA ACTGCATCAC AGAGATTTAC
1901 TATCAGTTCA AAAAAGACAA AGCAGAACGC AGACTAGCTT ATAATGAAGA
1951 ACAGATCCAC AAATTTGATA AGCAAAAATT GTATTACCAT GCCACAAAAG
2001 CAATGAGCCA CTTCTCAGAA GAATGTGTGA GAAAGTATGA AGCGTTTAAA
2051 GATAAGTCGG AAGAGTGGAT GAGAAAGATG CTTTCATCTTA GGAAGCAGCT
2101 GTTATCGCTA ACTAATCAGT GTTTCGATAT CGAAGAGGAA GTGTCCAAGT
2151 ATCAAGACTA TACTAACGAG TTACAAGAAA CTCTGCCTCA GAAAATGCTC
2201 GCAGCCTCCG GCGGCGTCAA GCACGCCATG GCCCCGATCT ACCCCAGCTC
2251 TAACACCTTA GTGGAGATGA CTCTTGGTAT GAAGAAGTTA AAGGAGGAGA
2301 TGGAAGGCGT GGTTAAGGAG CTGGCCGAGA ACAATCATAT TTTAGAAAGG
2351 TTTGGGTCTT TAACAATGGA TGGTGGCCTT CGCAATGTGG ACTGTCTTTA
2401 GCTTCCTAGG GAGTCTGGGA AGTTCTAGTT TGCACAAGAA GATAACACTG
2451 GGGCACGAAA TGAACACCTT TGTGAATGGA GTTCTTATTT CTACACTTCA
2501 GTATTTGATG AGGTCATGTA AATATGTACA GTTTGTAAAT ACATATACAT
2551 ATATATATAT ATATATGAAT TTTGGCTGCT GTAACAAAGA CAGATTGACC
2601 TCAGCGAGCT GTAGAAGAAA GCCATGACCA GCCAGTGCTT TGGGGTGCTC
2651 TCCCTAATTC TTCACATAAG GCTGGAGAAA TCAATTGCTT GGTGCCTAAA
2701 GAAAGTATTT TTTGAATTGG CATTCTTAAA ATTTTGAAAG GACTGATAGT
2751 CGACACAGTG TAACTGGAGG AGACACAGGG CTTTGTGACG GGAACAGAAC
2801 CGCGGTTTAA CCACAGTCGG TTCCCTGACA AGGATAAAGT GGCATTATCT
2851 CATTTGACCG GGTGCCCAA TCTCAGTTTT CCTCGGATGT TTGATTTTAG
2901 GTGAATTATT GAGCAAAAAC TTTAAAGTGA ATTCATTGTT TAAACTATTC
2951 ATTTTTCTT TGGTCATGAA TGTGTAATTG TCATTGAGAT CCTAGTATCA
3001 TTTCAATTGT CTTAAGATGT ATAATTTCTGT ACTTTAATTC TGCTATTTCA
3051 TGAAAAAATA AATTTCTCCC GGAATTCCTG CAGCCCGGGG GATCCACTAG
3101 TTCTAGAGCG GCCGCCACCG CGGTGGAGCT CCAGCTTTTG TTCCCTTTAG
3151 TGAGGGTTAA TTTCGAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT
3201 GTGAAATTGT TATCCGCTC (SEQ ID NO:3)

The amino acid sequences of the polypeptides encoded by the nucleotide sequence of the invention include:

Name: IKR-1 (Polypeptide)

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1  MQSTTNYLWH TDDLLGQGAT ASVYKARNKK SGEVVAVKVF NSASYRRPPE
51  VQVREFEVLRL RNHQNIVKL FAVEETGGSR QKVLIMEYCS SGSLLSVLED
101 PENTFGLSEE EFLVVLRCVV AGMNHLENG IVHRDIKPGN IMRLVGEEGQ
151 SIYKLSDFGA ARKLDDDEKF VSVYGTEEYL HPDMYERAVL RKPQQKAFGV
201 TVDLWSIGVT LYHAATGSLP FIPFGGPRRN KEIMYRITTE KPAGAISGTQ
251 KHENGPLEWS YSLPITCRLS MGLQNQLVPI LANILEVEED KCWGFDQFFA
301 ETSDILQRTV IHVFSLPQAV LHHVYIHAHN TIAIFLEAVY EQTNVTPKHQ
351 EYLFEGHPCV LEPSLSAQHI AHTAASSPLT LFSMSSDTPK GLAFRDPALD
401 VPKFVPKVDL QADYSTAKGV LGAGYQALWL ARVLLDGQAL MLRGLHWVLE
451 VLQDTCQQTL EVTRTALLYL SSSLGTERFS SGAGMPDVQE RKEATELRTR
501 LQTLSEILSK CSHNVTETQR SLSCLGEELL KNRDQIHEDN KSIQKIQCCL
551 DKMHFIYKQF KKSRRMRPGLS YNEEQIHKLD KVNFSHLAKR LLQVFQEECV
601 QTYQVSLVTH GKRMROVQRA QNHHLHIGHS VATCNSEARG AQESLNKIFD
651 QLLLDRASEQ GAEVSPQPMAPHPGDPKDL VFHMQELCND MKLLAFDLQD
701 NNRLIERLHR VPSAPDV (SEQ ID NO:2)

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Name: IKR-2 (Polypeptide)

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1  MQSTSNHLWL LSDILGQGAT ANVFRGRHKK TGDLYAVKVF NNISFLRPVD
51  VQVREFEVLK KLNHKNIVKL FAIEEETTTR HKVLIMEFCP CGSLYTVLEE
101 PSNAYGLPES EFLIVLRDVV GGMNHLENG IVHRDIKPGN IMRVIGEDGQ
151 SVYKLTDFGA ARELEDDEQF VSLYGTEEYL HPDMYERAVL RKDHQKKYGA
201 TVDLWSVGVT FYHAATGSLP FRPFEGPRRN KEVMYKIITG KPSGAISGVQ
251 KAENGPIDWS GDMPLSCSL S QGLQALLTPV LANILEADQE KCWGFDQFFA
301 ETSVDLHRMV IHVFSLQHMT AHKIYIHSYN TAAVFHELVEY KQTKIVSSNQ
351 ELIYEGRRLV LELGRLAQHF PKTTEENPIF VTSREQLNTV GLRYEKISLP
401 KIHPRYDLDG DASMAKAVTG VVCYACRTAS TLLLYQELMR KGVRWLVELV
451 KDDYNETVHK KTEVVITLDF CIRNIEKTVK VYEKLMKVNL EAAELGEISD
501 IHTKLLRLSS SQGTISSLQ DISSRLSPGG LLADTWAHQE GTHPRDRNVE
551 KLQVLLNCIT EIYYQFKKDK AERRLAYNEE QIHKFDKQKL YYHATKAMSH
601 FSEECVRKYE AFKDKSEEW RKMLHLRKQL LSLTNQCFDI EEEVSKYQDY
651 TNELQETLPQ KMLAASGGVK HAMAPIYPSS NTLVEMTLGM KKLKEEMEGV
701 VKELAENNIH LERFGSLTMD GGLRNVDCL (SEQ ID NO: 4)

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The discovery of the nucleic acids of the invention enables the construction of

expression vectors comprising nucleic acid sequences encoding polypeptides; host cells transfected or transformed with the expression vectors; isolated and purified biologically active polypeptides and fragments thereof. The invention also enables the use of the nucleic acids or oligonucleotides thereof as probes to identify nucleic acid encoding proteins having kinase function. The discovery further provides for the use of such polypeptides and soluble fragments to function as a kinase. The polypeptides and fragments can also generate antibodies, and among the uses of such antibodies is the purification of IKR polypeptides. Finally, the invention enables the use of such polypeptides and fragmented peptides as molecular weight markers and as controls for peptide fragmentation, as well as kits comprising these reagents.

NUCLEIC ACID MOLECULES

In a particular embodiment, the invention relates to certain isolated nucleotide sequences that are free from contaminating endogenous material. A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd sed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, *e.g.*, using the cDNA of SEQ ID NOs:1 and 2, or a suitable fragment thereof, as a probe.

The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. Other embodiments include DNA encoding a truncated version of the kinase containing, for example, only the kinase catalytic domain or a catalytically inactive mutant thereof.

Preferred Sequences

Particularly preferred nucleotide sequences of the invention are SEQ ID NOs:1 and 3, as set forth above. cDNA clones having the nucleotide sequence of SEQ ID NOs:1 and 3 were isolated as described in Example 1. Briefly, a mouse transit amplifying (TRAM) cell EST library was queried using the tBlastN algorithm to identify sequences related to KIAA0151 (GenBank accession number D63485), a kinase of unknown function which appears to be closely related to the I kappa B kinase (IKK) family of kinases. Query results yielded IKR-1 and IKR-2 ESTs which were then used to isolate IKR-1 and IKR-2 cDNA clones from the murine T-cell line bacteriophage library EL46.1N7ZAP.

IKR-1 and IKR-2 share 46% identity (56% similarity) to one another, whereas IKR-1 has 83% identity (87% similarity) with KIAA0151 and IKR-2 has 49% identity (58% similarity) with KIAA0151. Homologies of IKR-1 and IKR-2 toward the IKK family members are not as high as homologies to KIAA0151, generally in the range of 20% identity and 30% similarity.

IKR-2 also exhibits 94% identity to a recent entry (W60724) in the Derwent Geneseq database which describes a new protein kinase T2K that specifically phosphorylates I κ B.

The sequences of amino acids encoded by the DNA of SEQ ID NOs:1 and 3 are shown in SEQ ID NOs:2 and 4. These sequences identify the IKR polynucleotides as members of the I κ B kinase-related family. Members of this family such as IKK α and IKK β are activated in response to cytokine or other pro-inflammatory stimuli. Activated IKK α and IKK β specifically phosphorylate the inhibitory subunit I κ B (I κ B) α , which is bound to the transcription factor NF- κ B, at serine residues 32 and 36, thereby targeting this molecule for proteolytic destruction. Phosphorylation and destruction of I κ B results in the release of NF- κ B and its translocation from the cytosol to the nucleus where it engages transcriptional regulatory sites on a number of pro-inflammatory genes. The close similarity of the catalytic domains of IKR-1 and IKR-2 to the IKKs strongly suggest that they too will participate in the phosphorylation of I κ B (or related molecules) and lead to the activation of NF- κ B (or related transcription factors).

Additional Sequences

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NOs:1 and 3, and still encode a polypeptide having the amino acid sequence of SEQ ID NOs:2 and 4. Such variant DNA sequences can result from silent mutations (*e.g.*, occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA sequences encoding polypeptides of the

invention, selected from: (a) DNA comprising the nucleotide sequence of SEQ ID NOs:1 and 3; (b) DNA encoding the polypeptides of SEQ ID NOs:2 and 4 ; (c) DNA capable of hybridization to a DNA of (a) or (b) under conditions of moderate stringency and which encodes polypeptides of the invention; (d) DNA capable of hybridization to a DNA of (a) or (b) under conditions of high stringency and which encodes polypeptides of the invention, and (e) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b), (c), or (d) and which encode polypeptides of the invention. Of course, polypeptides encoded by such DNA sequences are encompassed by the invention.

As used herein, conditions of moderate stringency can be readily determined by those having ordinary skill in the art based on, for example, the length of the DNA. The basic conditions are set forth by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), and include use of a prewashing solution for the nitrocellulose filters 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, 6X SSC at about 42°C (or other similar hybridization solution, such as Stark's solution, in about 50% formamide at about 42°C), and washing conditions of about 60°C, 0.5X SSC, 0.1% SDS. Conditions of high stringency can also be readily determined by the skilled artisan based on, for example, the length of the DNA. Generally, such conditions are defined as hybridization conditions as above, and with washing at approximately 68°C, 0.2X SSC, 0.1% SDS. The skilled artisan will recognize that the temperature and wash solution salt concentration can be adjusted as necessary according to factors such as the length of the probe.

In another embodiment, the nucleic acid molecules of the invention also comprise nucleotide sequences that are at least 80% identical to a native sequence. Also contemplated are

embodiments in which a nucleic acid molecule comprises a sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to a native sequence.

The percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two nucleic acid sequences can be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention also provides isolated nucleic acids useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding an IKR-1 or IKR-2 polypeptide or desired fragment thereof may be subcloned into an expression vector for production of the polypeptide or fragment. The DNA sequence advantageously is fused to a sequence encoding a suitable leader or signal peptide. Alternatively, the desired fragment may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. If

necessary, oligonucleotides that reconstruct the 5' or 3' terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well-known polymerase chain reaction (PCR) procedure also may be employed to isolate and amplify a DNA sequence encoding a desired protein fragment. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified DNA fragment into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego (1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc. (1990).

POLYPEPTIDES AND FRAGMENTS THEREOF

The invention encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA technology. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

Polypeptides and Fragments Thereof

The polypeptides of the invention include full length proteins (amino acids 1 to 717 of SEQ ID NO:2 and amino acids 1 to 729 of SEQ ID NO:4) encoded by the nucleic acid sequences set forth above. Particularly preferred polypeptides comprise the amino acid sequence

of SEQ ID NOs:2 and 4 with particularly preferred fragments comprising the N-terminal kinase domain (amino acids 1 to 300) of SEQ ID NOs:2 and 4.

The invention also provides polypeptides and fragments of the kinase domain that retain a desired biological activity. Particular embodiments are directed to polypeptide fragments that retain the ability to bind "binding partners", native cognates, substrates, or counter-structures. Such a fragment may be a soluble polypeptide. In another embodiment, the polypeptides and fragments advantageously include regions that are conserved in the I kappa B kinase family as described above.

Also provided herein are polypeptide fragments comprising at least 20, or at least 30, contiguous amino acids of the sequence of SEQ ID NOs:2 and 4. Fragments derived from different domains find use in studies of signal transduction and in regulating cellular processes associated with transduction of biological signals. Polypeptide fragments also may be employed as immunogens, in generating antibodies.

Variants

Naturally occurring variants as well as derived variants of the polypeptides and fragments are provided herein.

Variants may exhibit amino acid sequences that are at least 80% identical. Also contemplated are embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof. Percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two protein sequences can be determined by comparing sequence

information using the GAP computer program, based on the algorithm of Needleman and Wunsch (J. Mol. Bio. 48:443, 1970) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a scoring matrix, blosum62, as described by Henikoff and Henikoff (Proc. Natl. Acad. Sci. USA 89:10915, 1992); (2) a gap weight of 12; (3) a gap length weight of 4; and (4) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The variants of the invention include, for example, those that result from alternate mRNA splicing events or from proteolytic cleavage. Alternate splicing of mRNA may, for example, yield a truncated but biologically active protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the protein (generally from 1-5 terminal amino acids). Proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

Additional variants within the scope of the invention include polypeptides that may be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared by linking the chemical moieties to functional groups on amino acid side chains or at the N-terminus or C-terminus of a polypeptide. Conjugates comprising diagnostic (detectable) or therapeutic agents attached thereto are within the scope of the invention.

Other derivatives include covalent or aggregative conjugates of the polypeptides with

other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG[®] peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Lys, which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG[®] peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG[®] peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. For example, these variants may exhibit differences in either kinase subdomain I or II. Kinase subdomain I has the consensus sequence "og-G-og-v", where uppercase letters represent invariant residues, lowercase letters represent highly conserved residues, "o" represents positions held by non polar residues, and "-" represents any amino acid. All lettering conforms to standard single letter code. "g" and "G" are Glycine and "v" is Valine. Kinase subdomain II has the consensus sequence "oaoK-o" where the same rules apply as described above, and "a" is Alanine and "K" is Lysine. Preferred variants are catalytically inactive variants of both IKR-1 and IKR-2 in which, for example, the invariant

lysine residue (amino acid 38 in SEQ ID NOs:2 and 4) present in kinase subdomain II in the active site of functional kinases is substituted for an arginine or alanine residue. Additional variants encompass the substitution of invariant glycine residue (amino acid 18 in SEQ ID NOs:2 and 4) present in kinase subdomain I in the ATP binding site of kinases for any other residue.

Other particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.

A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other conservative substitutions, *e.g.*, involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, the DNAs of the invention include variants that differ from a native DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide.

In another example of variants, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation.

Other variants are prepared by modification of adjacent dibasic amino acid residues,

to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

PRODUCTION OF POLYPEPTIDES AND FRAGMENTS THEREOF

Expression, isolation and purification of the polypeptides and fragments of the invention may be accomplished by any suitable technique, including but not limited to the following:

Expression Systems

The present invention also provides recombinant cloning and expression vectors containing DNA, as well as host cell containing the recombinant vectors. Expression vectors comprising DNA may be used to prepare the polypeptides or fragments of the invention encoded by the DNA. A method for producing polypeptides comprises culturing host cells transformed with a recombinant expression vector encoding the polypeptide, under conditions that promote expression of the polypeptide, then recovering the expressed polypeptides from the culture. The skilled artisan will recognize that the procedure for purifying the expressed polypeptides will vary according to such factors as the type of host cells employed and any particular characteristics of the polypeptide.

Any suitable expression system may be employed. The vectors include a DNA encoding a polypeptide or fragment of the invention, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA sequence. Thus, a promoter nucleotide sequence is operably linked to a DNA sequence if the promoter nucleotide sequence controls the transcription of the DNA sequence. An origin of replication that confers the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the nucleic acid sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide upon secretion of polypeptide from the cell.

The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved may differ from that predicted by computer program, and may vary according to such factors as the type of host cells employed in expressing a recombinant polypeptide. A protein preparation may include a mixture of protein molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site.

Suitable host cells for expression of polypeptides include prokaryotes, yeast or higher eukaryotic cells. Mammalian or insect cells are generally preferred for use as host cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotic Systems

Prokaryotes include gram-negative or gram-positive organisms. Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and

Staphylococcus. In a prokaryotic host cell, such as *E. coli*, a polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β -lactamase (penicillinase), lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EP-A-36776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λP_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λP_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC

53082).

Yeast Systems

Alternatively, the polypeptides may be expressed in yeast host cells, preferably from the *Saccharomyces* genus (e.g., *S. cerevisiae*). Other genera of yeast, such as *Pichia* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2 μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phospho-glucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α -factor leader sequence may be employed to direct secretion of the polypeptide. The α -factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. See, e.g., Kurjan et al., *Cell* 30:933, 1982 and Bitter et al., *Proc.*

Natl. Acad. Sci. USA 81:5330, 1984. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978. The Hinnen et al. protocol selects for Trp⁺ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 mg/ml adenine and 20 mg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 mg/ml adenine and 80 mg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or Insect Systems

Mammalian or insect host cell culture systems also may be employed to express recombinant polypeptides. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell* 23:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line

derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture*, 1990, pp. 15-69). Additional protocols using commercially available reagents, such as Lipofectamine lipid reagent (Gibco/BRL) or Lipofectamine-Plus lipid reagent, can be used to transfect cells (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1987). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman et al., *Meth. in Enzymology* 185:487-511, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be incorporated into an expression vector include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

Transcriptional and translational control sequences for mammalian host cell expression vectors can be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example,

SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers et al., *Nature* 273:113, 1978; Kaufman, *Meth. in Enzymology*, 1990). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris et al., *Animal Cell Technology*, 1997, pp. 529-534 and PCT Application WO 97/25420) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras et al., *J. Biol. Chem.* 257:13475-13491, 1982). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, *Current Opinion in Genetics and Development* 3:295-300, 1993; Ramesh et al., *Nucleic Acids Research* 24:2697-2700, 1996). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for a selectable marker (e.g. DHFR) has been shown to improve transfectability of the host and expression of the heterologous cDNA (Kaufman, *Meth. in Enzymology*, 1990). Exemplary expression vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser et al., *Biotechniques* 22:150-161, 1997, and p2A5I described by Morris et al., *Animal Cell Technology*, 1997, pp. 529-534.

A useful high expression vector, pCAVNOT, has been described by Mosley et al., *Cell* 59:335-348, 1989. Other expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). A useful system

for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature* 312:768, 1984, has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in WO 91/18982, incorporated by reference herein. In yet another alternative, the vectors can be derived from retroviruses.

Additional useful expression vectors, pFLAG[®] and pDC311, can also be used. FLAG[®] technology is centered on the fusion of a low molecular weight (1kD), hydrophilic, FLAG[®] marker peptide to the N-terminus of a recombinant protein expressed by pFLAG[®] expression vectors. pDC311 is another specialized vector used for expressing proteins in CHO cells. pDC311 is characterized by a bicistronic sequence containing the gene of interest and a dihydrofolate reductase (DHFR) gene with an internal ribosome binding site for DHFR translation, an expression augmenting sequence element (EASE), the human CMV promoter, a tripartite leader sequence, and a polyadenylation site.

Regarding signal peptides that may be employed, the choice of signal peptide or leader may depend on factors such as the type of host cells in which the recombinant polypeptide is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

Purification

The invention also includes methods of isolating and purifying the polypeptides and fragments thereof.

Isolation and Purification

The "isolated" polypeptides or fragments thereof encompassed by this invention are polypeptides or fragments that are not in an environment identical to an environment in which it or they can be found in nature. The "purified" polypeptides or fragments thereof encompassed by this invention are essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant expression systems such as those described above or as a purified product from a non-recombinant source such as naturally occurring cells and/or tissues.

In one preferred embodiment, the purification of recombinant polypeptides or fragments can be accomplished using fusions of polypeptides or fragments of the invention to another polypeptide to aid in the purification of polypeptides or fragments of the invention. Such fusion partners can include the poly-His or other antigenic identification peptides described above.

With respect to any type of host cell, as is known to the skilled artisan, procedures for purifying a recombinant polypeptide or fragment will vary according to such factors as the type of host cells employed and whether or not the recombinant polypeptide or fragment is secreted into the culture medium.

In general, the recombinant polypeptide or fragment can be isolated from the host cells if not secreted, or from the medium or supernatant if soluble and secreted, followed by one

or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification or size exclusion chromatography steps. As to specific ways to accomplish these steps, the culture medium first can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In addition, a chromatofocusing step can be employed. Alternatively, a hydrophobic interaction chromatography step can be employed. Suitable matrices can be phenyl or octyl moieties bound to resins. In addition, affinity chromatography with a matrix which selectively binds the recombinant protein can be employed. Examples of such resins employed are lectin columns, dye columns, and metal-chelating columns. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel or polymer resin having pendant methyl, octyl, octyldecyl or other aliphatic groups) can be employed to further purify the polypeptides. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide an isolated and purified recombinant protein.

It is also possible to utilize an affinity column comprising a polypeptide-binding protein of the invention, such as a monoclonal antibody generated against polypeptides of the invention, to affinity-purify expressed polypeptides. These polypeptides can be removed from

an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized, or be competitively removed using the naturally occurring substrate of the affinity moiety, such as a polypeptide derived from the invention.

In this aspect of the invention, polypeptide-binding proteins, such as the anti-polypeptide antibodies of the invention or other proteins that may interact with the polypeptide of the invention, can be bound to a solid phase support such as a column chromatography matrix or a similar substrate suitable for identifying, separating, or purifying cells that express polypeptides of the invention on their surface. Adherence of polypeptide-binding proteins of the invention to a solid phase contacting surface can be accomplished by any means, for example, magnetic microspheres can be coated with these polypeptide-binding proteins and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid phase that has such polypeptide-binding proteins thereon. Cells having polypeptides of the invention on their surface bind to the fixed polypeptide-binding protein and unbound cells then are washed away. This affinity-binding method is useful for purifying, screening, or separating such polypeptide-expressing cells from solution. Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner.

Alternatively, mixtures of cells suspected of containing polypeptide-expressing cells of the invention first can be incubated with a biotinylated polypeptide-binding protein of the invention. Incubation periods are typically at least one hour in duration to ensure sufficient binding to polypeptides of the invention. The resulting mixture then is passed through a column

packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. See Berenson, et al. *J. Cell. Biochem.*, 10D:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the polypeptide is to be administered *in vivo*, for example. In such a case, the polypeptides are purified such that no protein bands corresponding to other proteins are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to the polypeptide may be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. Most preferably, the polypeptide of the invention is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

USE OF IKR NUCLEIC ACID OR OLIGONUCLEOTIDES

In addition to being used to express polypeptides as described above, the nucleic acids of the invention, including DNA and oligonucleotides thereof, can be used as probes to identify other nucleic acids encoding proteins having kinase activity.

Among the uses of nucleic acids of the invention is the use of fragments thereof as probes or primers to aid in the isolation of other proteins having kinase activity. Such fragments generally comprise at least about 17 contiguous nucleotides of a DNA sequence. In other embodiments, a DNA fragment comprises at least 30, or at least 60, contiguous nucleotides of a

DNA sequence.

Because homologs of SEQ ID NOS:1 and 2, from other mammalian species are contemplated herein, probes based on the murine DNA sequence of SEQ ID NOS:1 and 2 may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

Using knowledge of the genetic code in combination with the amino acid sequences set forth above, sets of degenerate oligonucleotides can be prepared. Such oligonucleotides are useful as primers, e.g., in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified.

USE OF IKR POLYPEPTIDES AND FRAGMENTED POLYPEPTIDES

Uses include, but are not limited to, the following:

- Purifying proteins and measuring activity thereof
- Research Reagents
- Molecular weight and Isoelectric focusing markers
- Controls for peptide fragmentation
- Identification of unknown proteins
- Preparation of Antibodies

Purification Reagents

Each of the polypeptides of the invention finds use as a protein purification reagent. For example, the polypeptides may be used to purify binding partner proteins, such as naturally occurring substrates or protein inhibitors, which bind specifically to the catalytic site of either

IKR-1 or IKR-2. In addition, both IKR-1 and IKR-2 might also serve as substrates for other kinases in a kinase cascade, such as kinases that bind to and modulate the activity of or cellular localization of IKR-1 or IKR-2. Therefore they might be used to bind and facilitate purification of these other kinases and proteins.

In particular embodiments, a polypeptide (in any form described herein that is capable of binding a binding partner) is attached to a solid support by conventional procedures. As one example, affinity chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). In an alternative, a polypeptide/Fc protein (as discussed above) is attached to Protein A- or Protein G-containing chromatography columns through interaction with the Fc moiety.

Measuring Activity

Polypeptides also find use in measuring the biological activity of binding partner proteins in terms of their binding affinity. The polypeptides thus may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of protein under different conditions. For example, the polypeptides may be employed in a binding affinity study to measure the biological activity of a binding partner protein that has been stored at different temperatures, or produced in different cell types. The proteins also may be used to determine whether biological activity is retained after modification of a binding partner protein (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified binding partner protein is compared to that of an unmodified binding partner protein to detect any adverse impact of the modifications on biological activity of the binding partner. The biological

activity of a binding partner protein thus can be ascertained before it is used in a research study, for example.

In a particularly preferred embodiment, the polypeptides, fragments or fusion proteins thereof can be used to assay protein kinase activity.

Research Agents

Polypeptides of the invention may be used to set up screening assays to identify molecules from, for example, chemical libraries, combinatorial chemistry libraries, natural product libraries or other libraries of small organic molecules that inhibit or activate IKR-1 and/or IKR-2 activity. Such a small molecule antagonist or agonist could be useful in disrupting or enhancing molecular signaling to certain transcriptional activators.

Another embodiment of the invention is the use of isolated IKR-1 or IKR-2 polypeptides, fusion proteins, or a fragment thereof containing the isolated protein kinase domain of IKR-1 or IKR-2 in *in vitro* or *in vivo* assays to determine protein kinase activity. A hallmark of protein kinases is their ability to phosphorylate other proteins and to auto-phosphorylate. Therefore, in one aspect of the invention, the isolated polypeptides with kinase activity can be used in assays to phosphorylate target proteins, radiolabel target proteins with ^{32}P , and identify proteins having phosphatase activity. Exemplary methods of phosphorylation assays set forth above are disclosed in U.S. Patents 5,447,860 which is incorporated herein by reference. In addition to full length polypeptides, the invention also includes the isolated active kinase domains of kinases which can function as reagents in kinase assays.

Kinase assays are typically carried out by combining IKR-1, IKR-2, or an active kinase domain with radiolabeled ATP ($\gamma^{32}\text{P}$ -ATP) and a peptide or protein substrate in a buffer solution.

The peptide substrates generally range from 8 to 30 amino acids in length or the substrate may also be a protein known to be phosphorylated readily by IKR-1 and/or IKR-2. Many such general kinase substrates are known, such as α or β casein, histone H1, myelin basic protein, etc. After incubation of this reaction mixture at 20-37°C for a suitable time, the kinase-mediated transfer of radioactive phosphate from ATP to the substrate protein or substrate peptide can be determined by methods well known in the art.

The purpose of such an assay would be to identify substances which interfere with the rate of substrate phosphorylation. Such inhibitory substances could serve as lead compounds in the development of pharmaceuticals for the treatment of autoimmune, inflammatory, infectious or neoplastic diseases in which there is a dysregulation of the inflammatory processes mediated by IKR-1 or IKR-2. It is conceivable that compounds which inhibit IKR-1 or IKR-2 could have merit as more general inhibitors of the class of protein kinases which mediate the inflammatory process, including (but not limited to) those mentioned above.

Yet another embodiment of the invention relates to the use of IKR polypeptides and fragments as reagents to identify (a) any protein that the polypeptide regulates, and (b) other proteins with which it might interact. Thus, IKR-1 and IKR-2 can be used to study, for example, cell signal transduction.

The IKRs, like other I kappa B kinases, could play a central role in immune responses which includes cellular signal transduction and inflammatory responses. As such, alterations in the expression and/or activation of IKR-1 and/or IKR-2 can have profound effects on a plethora of cellular processes. Expression of cloned IKR-1 and/or IKR-2, functionally inactive mutants of IKR-1 and/or IKR-2, or the kinase domain can be used to identify the role a particular protein

plays in mediating specific signaling events.

Cellular signaling often involves a molecular activation cascade, during which a receptor propagates a ligand-receptor mediated signal by specifically activating intracellular kinases which phosphorylate target substrates, ultimately resulting in the activation of the transcription factor NF κ B and/or other transcription factors. These substrates can themselves be kinases which become activated following phosphorylation. Alternatively, they can be adaptor molecules that facilitate down stream signaling through protein-protein interaction following phosphorylation. Regardless of the nature of the substrate molecule(s), expressed functionally-active versions of IKR-1 and/or IKR-2, for example the IKR kinase domain, can be used in assays such as the yeast 2-hybrid assay to identify what substrate(s) were recognized and altered by IKR-1 and/or IKR-2. As such, these novel IKR-1 or IKR-2 polypeptides can be used as reagents to identify novel molecules involved in signal transduction pathways. In addition, IKR-1, IKR-2, and other downstream molecules involved in the signal transduction pathway can be potential targets for therapeutic compounds that interfere with the activation of NF- κ B.

Molecular Weight, Isoelectric Point Markers

The polypeptides of the present invention can be subjected to fragmentation into smaller peptides by chemical and enzymatic means, and the peptide fragments so produced can be used in the analysis of other proteins or polypeptides. For example, such peptide fragments can be used as peptide molecular weight markers, peptide isoelectric point markers, or in the analysis of the degree of peptide fragmentation. Thus, the invention also includes these polypeptides and peptide fragments, as well as kits to aid in the determination of the apparent molecular weight and isoelectric point of an unknown protein and kits to assess the degree of fragmentation of an

unknown protein.

Although all methods of fragmentation are encompassed by the invention, chemical fragmentation is a preferred embodiment, and includes the use of cyanogen bromide to cleave under neutral or acidic conditions such that specific cleavage occurs at methionine residues (E. Gross, *Methods in Enz.* 11:238-255, 1967). This can further include additional steps, such as a carboxymethylation step to convert cysteine residues to an unreactive species.

Enzymatic fragmentation is another preferred embodiment, and includes the use of a protease such as Asparaginylendo-peptidase, Arginylendo-peptidase, *Achromobacter* protease I, Trypsin, *Staphylococcus aureus* V8 protease, Endoproteinase Asp-N, or Endoproteinase Lys-C under conventional conditions to result in cleavage at specific amino acid residues.

Asparaginylendo-peptidase can cleave specifically on the carboxyl side of the asparagine residues present within the polypeptides of the invention. Arginylendo-peptidase can cleave specifically on the carboxyl side of the arginine residues present within these polypeptides. *Achromobacter* protease I can cleave specifically on the carboxyl side of the lysine residues present within the polypeptides (Sakiyama and Nakat, U.S. Patent No. 5,248,599; T. Masaki et al., *Biochim. Biophys. Acta* 660:44-50, 1981; T. Masaki et al., *Biochim. Biophys. Acta* 660:51-55, 1981). Trypsin can cleave specifically on the carboxyl side of the arginine and lysine residues present within polypeptides of the invention. Enzymatic fragmentation may also occur with a protease that cleaves at multiple amino acid residues. For example, *Staphylococcus aureus* V8 protease can cleave specifically on the carboxyl side of the aspartic and glutamic acid residues present within polypeptides (D. W. Cleveland, *J. Biol. Chem.* 3:1102-1106, 1977). Endoproteinase Asp-N can cleave specifically on the amino side of the asparagine residues present within polypeptides. Endoproteinase Lys-C can cleave specifically on the carboxyl side

of the lysine residues present within polypeptides of the invention. Other enzymatic and chemical treatments can likewise be used to specifically fragment these polypeptides into a unique set of specific peptides.

Of course, the peptides and fragments of the polypeptides of the invention can also be produced by conventional recombinant processes and synthetic processes well known in the art. With regard to recombinant processes, the polypeptides and peptide fragments encompassed by invention can have variable molecular weights, depending upon the host cell in which they are expressed.

The molecular weight of these polypeptides can also be varied by fusing additional peptide sequences to both the amino and carboxyl terminal ends of polypeptides of the invention. Fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention can be used to enhance expression of these polypeptides or aid in the purification of the protein. In addition, fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention will alter some, but usually not all, of the fragmented peptides of the polypeptides generated by enzymatic or chemical treatment. Of course, mutations can be introduced into polypeptides of the invention using routine and known techniques of molecular biology. For example, a mutation can be designed so as to eliminate a site of proteolytic cleavage by a specific enzyme or a site of cleavage by a specific chemically induced fragmentation procedure. The elimination of the site will alter the peptide fingerprint of polypeptides of the invention upon fragmentation with the specific enzyme or chemical procedure.

Because the unique amino acid sequence of each piece specifies a molecular weight, these pieces can thereafter serve as molecular weight markers using such analysis techniques to

assist in the determination of the molecular weight of an unknown protein, polypeptides or fragments thereof. The molecular weight markers of the invention serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of proteins that have similar apparent molecular weights and, consequently, allow increased accuracy in the determination of apparent molecular weight of proteins.

When the invention relates to the use of fragmented peptide molecular weight markers, those markers are preferably at least 10 amino acids in size. More preferably, these fragmented peptide molecular weight markers are between 10 and 100 amino acids in size. Even more preferable are fragmented peptide molecular weight markers between 10 and 50 amino acids in size and especially between 10 and 35 amino acids in size. Most preferable are fragmented peptide molecular weight markers between 10 and 20 amino acids in size.

Among the methods for determining molecular weight are sedimentation, gel electrophoresis, chromatography, and mass spectrometry. A particularly preferred embodiment is denaturing polyacrylamide gel electrophoresis (U. K. Laemmli, *Nature* 227:680-685, 1970). Conventionally, the method uses two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 6-20%. The ability to simultaneously resolve the marker and the sample under identical conditions allows for increased accuracy. It is understood, of course, that many different techniques can be used for the determination of the molecular weight of an unknown protein using polypeptides of the invention, and that this embodiment in no way limits the scope of the invention.

Each polypeptide or fragment thereof has a pI that is intrinsically determined by its unique amino acid sequence (which pI can be estimated by the skilled artisan using any of the computer programs designed to predict pI values currently available, calculated using any well-

known amino acid pKa table, or measured empirically). Therefore these polypeptides and fragments thereof can serve as specific markers to assist in the determination of the isoelectric point of an unknown protein, polypeptide, or fragmented peptide using techniques such as isoelectric focusing. These polypeptide or fragmented peptide markers serve particularly well for the estimation of apparent isoelectric points of unknown proteins that have apparent isoelectric points close to that of the polypeptide or fragmented peptide markers of the invention.

The technique of isoelectric focusing can be further combined with other techniques such as gel electrophoresis to simultaneously separate a protein on the basis of molecular weight and charge. The ability to simultaneously resolve these polypeptide or fragmented peptide markers and the unknown protein under identical conditions allows for increased accuracy in the determination of the apparent isoelectric point of the unknown protein. This is of particular interest in techniques, such as two dimensional electrophoresis (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)), where the nature of the procedure dictates that any markers should be resolved simultaneously with the unknown protein. In addition, with such methods, these polypeptides and fragmented peptides thereof can assist in the determination of both the isoelectric point and molecular weight of an unknown protein or fragmented peptide.

Polypeptides and fragmented peptides can be visualized using two different methods that allow a discrimination between the unknown protein and the molecular weight markers. In one embodiment, the polypeptide and fragmented peptide molecular weight markers of the invention can be visualized using antibodies generated against these markers and conventional immunoblotting techniques. This detection is performed under conventional conditions that do not result in the detection of the unknown protein. It is understood that it may not be possible to

generate antibodies against all polypeptide fragments of the invention, since small peptides may not contain immunogenic epitopes. It is further understood that not all antibodies will work in this assay; however, those antibodies which are able to bind polypeptides and fragments of the invention can be readily determined using conventional techniques.

The unknown protein is also visualized by using a conventional staining procedure. The molar excess of unknown protein to polypeptide or fragmented peptide molecular weight markers of the invention is such that the conventional staining procedure predominantly detects the unknown protein. The level of these polypeptide or fragmented peptide molecular weight markers is such as to allow little or no detection of these markers by the conventional staining method. The preferred molar excess of unknown protein to polypeptide molecular weight markers of the invention is between 2 and 100,000 fold. More preferably, the preferred molar excess of unknown protein to these polypeptide molecular weight markers is between 10 and 10,000 fold and especially between 100 and 1,000 fold.

It is understood of course that many techniques can be used for the determination and detection of molecular weight and isoelectric point of an unknown protein, polypeptides, and fragmented peptides thereof using these polypeptide molecular weight markers and peptide fragments thereof and that these embodiments in no way limit the scope of the invention.

In another embodiment, the analysis of the progressive fragmentation of the polypeptides of the invention into specific peptides (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977), such as by altering the time or temperature of the fragmentation reaction, can be used as a control for the extent of cleavage of an unknown protein. For example, cleavage of the same amount of polypeptide and unknown protein under identical conditions can allow for a direct comparison of the extent of fragmentation. Conditions that result in the complete fragmentation

of the polypeptide can also result in complete fragmentation of the unknown protein.

As to the specific use of the polypeptides and fragmented peptides of the invention as molecular weight markers, the fragmentation of the polypeptide of SEQ ID NOs:3 and 4 with cyanogen bromide generates a unique set of fragmented peptide molecular weight markers with the molecular weights shown in Table I. The distribution of methionine residues determines the number of amino acids in each peptide and the unique amino acid composition of each peptide determines its molecular weight.

Table I

Cleavage of IKR-1 and IKR-2 with cyanogen bromide

IKR-1			IKR-2		
Fragment #	Residues	Avg. MW	Fragment #	Residues	Avg. MW
1	1-1	101.10	1	1-1	101.10
2	685-691	803.85	2	687-690	372.42
3	554-565	1564.85	3	621-623	385.47
4	670-684	1609.81	4	691-697	857.02
5	124-142	2165.45	5	663-673	993.13
6	670-691	2443.75	6	720-729	1061.19
7	692-717	3030.49	7	310-319	1162.36
8	685-717	3864.43	8	687-697	1259.53
9	87-123	3975.47	9	674-686	1373.52
10	235-271	4088.61	10	674-690	1776.04
11	143-184	4761.19	11	124-142	2165.45
12	185-234	5569.46	12	663-686	2396.75
13	566-614	5751.57	13	698-719	2438.73
14	385-441	6027.92	14	415-439	2657.14
15	615-669	6032.69	15	599-620	2717.95
16	87-142	6171.01	16	599-623	3133.51
17	124-184	6956.73	17	691-719	3325.84
18	554-614	7346.52	18	698-729	3530.01
19	615-684	7672.59	19	54-86	3960.69
20	2-86	9613.92	20	87-123	3987.52
21	185-271	9688.16	21	624-662	4679.24
IKR-1			IKR-2		

Fragment #	Residues	Avg. MW	Fragment #	Residues	Avg. MW
22	1-86	9745.12	22	143-184	4776.11
23	143-234	10360.74	23	621-662	5094.80
24	566-669	11814.35	24	440-486	5632.61
25	272-384	12699.43	25	624-673	5702.47
26	442-553	12715.48	26	185-234	5762.53
27	2-123	13619.48	27	2-53	5796.60
28	442-565	14310.42	28	1-53	5927.80
29	235-384	16818.13	29	87-142	6183.06
30	272-441	18757.45	30	124-184	6971.66
31	385-553	18773.50	31	54-123	7978.30
			32	235-309	8078.16
			33	415-486	8319.85
			34	235-319	9270.61
			35	2-86	9787.38
			36	143-234	10568.74
			37	320-414	10981.47
			38	310-414	12173.92
			39	487-598	12852.49
			40	320-439	13668.70
			41	185-309	13870.79
			42	487-620	15600.53
			43	440-598	18515.20

In addition, the preferred purified polypeptides of the invention (SEQ ID NOs:3 and 4) have a calculated molecular weight of approximately 80,962 and 83,425 Daltons, respectively, and use thereof allows increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 80,962 or 83,425 Daltons.

Finally, as to the kits that are encompassed by the invention, the constituents of such kits can be varied, but typically contain the polypeptide and fragmented peptide molecular weight markers. Also, such kits can contain the polypeptides wherein a site necessary for fragmentation has been removed. Furthermore, the kits can contain reagents for the specific cleavage of the polypeptide and the unknown protein by chemical or enzymatic cleavage. Kits can further contain antibodies directed against polypeptides or fragments thereof of the invention.

Identification of Unknown Proteins

As set forth above, a polypeptide or peptide fingerprint can be entered into or compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., Proc. Natl. Acad. Sci. USA 90:5011-5015, 1993; D. Fenyo et al., Electrophoresis 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearchForm.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare observed molecular weights to predicted peptide molecular weights derived from

sequence databases to assist in determining the identity of the unknown protein.

In addition, a polypeptide or peptide digest can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., J. Am. Soc. Mass Spec. 5:976-989 (1994); M. Mann and M. Wilm, Anal. Chem. 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, Rapid Comm. Mass Spec. 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site: www.lsbc.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search and ProFound programs described above.

Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using mass spectrometry.

Antibodies

Antibodies that are immunoreactive with the polypeptides of the invention are provided herein. Such antibodies specifically bind to the polypeptides *via* the antigen-binding sites of the antibody (as opposed to non-specific binding). Thus, the polypeptides, fragments, variants, fusion proteins, etc., as set forth above may be employed as "immunogens" in producing antibodies immunoreactive therewith. More specifically, the polypeptides, fragment, variants, fusion proteins, etc. contain antigenic determinants or epitopes that elicit the formation of antibodies.

These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids

sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 3:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the protein and steric hinderances, the number of antibodies that actually bind to the epitopes is less than the number of available epitopes (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 2:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes may be identified by any of the methods known in the art.

Thus, one aspect of the present invention relates to the antigenic epitopes of the polypeptides of the invention. Such epitopes are useful for raising antibodies, in particular monoclonal antibodies, as described in more detail below. Additionally, epitopes from the polypeptides of the invention can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques well known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

As to the antibodies that can be elicited by the epitopes of the polypeptides of the invention, whether the epitopes have been isolated or remain part of the polypeptides, both polyclonal and monoclonal antibodies may be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*,

Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

Hybridoma cell lines that produce monoclonal antibodies specific for the polypeptides of the invention are also contemplated herein. Such hybridomas may be produced and identified by conventional techniques. One method for producing such a hybridoma cell line comprises immunizing an animal with a polypeptide; harvesting spleen cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds the polypeptide. The monoclonal antibodies may be recovered by conventional techniques.

The monoclonal antibodies of the present invention also include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature* 332:323, 1988), Liu et al. (*PNAS* 84:3439, 1987), Larrick et al. (*Bio/Technology* 7:934, 1989), and Winter and Harris (*TIPS* 14:139, May, 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, US Patent Nos. 5,569,825 and

5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein.

Antigen-binding fragments of the antibodies, which may be produced by conventional techniques, are also encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab and F(ab')₂ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

In one embodiment, the antibodies are specific for the polypeptides of the present invention and do not cross-react with other proteins. Screening procedures by which such antibodies may be identified are well known, and may involve immunoaffinity chromatography, for example.

Uses Thereof

The antibodies of the invention can be used in assays to detect the presence of the polypeptides or fragments of the invention, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying polypeptides or fragments of the invention by immunoaffinity chromatography.

Those antibodies that additionally can block binding of the polypeptides of the invention to the binding partner(s) may be used to inhibit a biological activity that results from such binding. Such blocking antibodies may be identified using any suitable assay procedure, such as by testing antibodies for the ability to inhibit binding of IKR-1 and/or IKR-2 to the binding partner(s). Alternatively, blocking antibodies may be identified in assays for the ability to inhibit a biological effect that results from binding of IKR-1 and/or IKR-2 to its binding partner(s).

Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a biological activity mediated by the entity that generated the antibody. Disorders caused or exacerbated (directly or indirectly) by the interaction of IKR-1 and/or IKR-2 with a binding partner thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody in an intracellular form to a mammal in an amount effective in inhibiting an IKR-1 and/or IKR-2-mediated biological activity. Monoclonal antibodies are generally preferred for use in such therapeutic methods.

Antibodies may be screened for agonistic (*i.e.*, ligand-mimicking) properties. Such antibodies, upon binding to IKR-1 and/or IKR-2 binding partner(s), induce biological effects (e.g., transduction of biological signals) similar to the biological effects induced when IKR-1 or IKR-2 binds to a binding partner.

Furthermore, antibodies may be used to specifically immunoprecipitate IKR-1 and/or IKR-2. Such immunoprecipitates can be captured by procedures known in the art, such as binding to resin attached Protein A. The resultant resin bound complex can be utilized as a source of kinase for *in vitro* kinase ^{32}P phosphotransferase assays as described above.

Compositions comprising an antibody that is directed against IKR-1 and/or IKR-2, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing IKR-1, IKR-2, IKR-1 binding partner proteins, or IKR-2 binding partner proteins.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to the antibody. Examples of such agents are presented above. The conjugates find use in *in vitro* or *in vivo* procedures.

The following examples are provided to further illustrate particular embodiments of the

invention, and are not to be construed as limiting the scope of the present invention.

EXAMPLE 1: Isolation of the Nucleic Acid

The original cDNA clone from which the sequences are derived was obtained from a murine transit amplifying cell (TRAM) library that is maintained by *Genesis*. Independent clones for both IKR-1 and IKR-2 were obtained from the murine T-cell line bacteriophage library, EL46.1N7ZAP. In the case of IKR-1, three independent clones were isolated and required to obtain full length sequence, whereas a single full length IKR-2 clone was isolated. Full length sequences for the novel protein kinases IKR-1 (SEQ ID NO:1) and IKR-2 (SEQ ID NO:3) are shown above.

IKR-1 (SEQ ID NO:1) contains 5' and 3' untranslated sequence and the coding region consisting of nucleotides 369-2520, and IKR-2 (SEQ ID NO:3) also contains 5' and 3' untranslated sequence and the coding region consisting of nucleotides 212-2398. SEQ ID NOs:2 and 4 show translated full length open reading frames for IKR-1 and IKR-2, respectively.

EXAMPLE 2: Use of IKR polypeptides in an ELISA

Serial dilutions of IKR-containing samples (in 50 mM NaHCO₃, brought to pH 9 with NaOH) are coated onto Linbro/Titertek 96 well flat bottom E.I.A. microtitration plates (ICN Biomedicals Inc., Aurora, OH) at 100:1/well. After incubation at 4°C for 16 hours, the wells are washed six times with 200:1 PBS containing 0.05% Tween-20 (PBS-Tween). The wells are then incubated with FLAG®-IKR binding partner at 1 µg/ml in PBS-Tween with 5% fetal calf serum (FCS) for 90 minutes (100:1 per well), followed by washing as above. Next, each well is incubated with the anti-FLAG® (monoclonal antibody M2 at 1 µg/ml in PBS-Tween containing

5% FCS for 90 minutes (100:1 per well), followed by washing as above. Subsequently, wells are incubated with a polyclonal goat anti-mIgG1-specific horseradish peroxidase-conjugated antibody (a 1:5000 dilution of the commercial stock in PBS-Tween containing 5% FCS) for 90 minutes (100:1 per well). The HRP-conjugated antibody is obtained from Southern Biotechnology Associates, Inc., Birmingham, Alabama. Wells then are washed six times, as above.

For development of the ELISA, a substrate mix [100:1 per well of a 1:1 premix of the TMB Peroxidase Substrate and Peroxidase Solution B (Kirkegaard Perry Laboratories, Gaithersburg, Maryland)] is added to the wells. After sufficient color reaction, the enzymatic reaction is terminated by addition of 2 N H₂SO₄ (50 :1 per well). Color intensity (indicating IKR-1 and/or IKR-2-binding activity) is determined by measuring extinction at 450 nm on a V Max plate reader (Molecular Devices, Sunnyvale, CA).

EXAMPLE 3: Monoclonal Antibodies That Bind

This example illustrates a method for preparing monoclonal antibodies that bind IKR-1 and/or IKR-2 polypeptides. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified IKR-1 and/or IKR-2 polypeptides or an immunogenic fragment thereof.

Purified IKR-1 and/or IKR-2 can be used to generate monoclonal antibodies immunoreactive therewith, using conventional techniques such as those described in U.S. Patent 4,411,993. Briefly, mice are immunized with IKR-1 and/or IKR-2 immunogen emulsified in complete Freund's adjuvant, and injected in amounts ranging from 10-100 :g subcutaneously or intraperitoneally. Ten to twelve days later, the immunized animals are boosted with additional

IKR-1 and/or IKR-2 emulsified in incomplete Freund's adjuvant. Mice are periodically boosted thereafter on a weekly to bi-weekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for IKR-1 and/or IKR-2 antibodies by dot blot assay, ELISA (Enzyme-Linked Immunosorbent Assay), inhibition of IKR-1 and/or IKR-2-binding partner binding, or inhibition of kinase catalytic activity.

Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of IKR-1 and/or IKR-2 in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, e.g., NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened by ELISA for reactivity against purified IKR-1 and/or IKR-2 by adaptations of the techniques disclosed in Engvall et al., *Immunochem.* 8:871, 1971 and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol.* 144:4212, 1990). Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB/c mice to produce ascites containing high concentrations of anti-IKR-1 and/or IKR-2 monoclonal antibodies. Alternatively, hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to Protein A or Protein G can also be used, as can affinity chromatography based upon binding to IKR-1 and/or IKR-2.

EXAMPLE 4: Northern Blot Analysis

The tissue distribution of IKR-1 and IKR-2 mRNA can be investigated by Northern blot analysis, as follows. An aliquot of a radiolabeled probe (^{32}P -labeled PCR product derived from IKR-1 or IKR-2 specific oligonucleotide primers) is added to murine and human multiple tissue Northern blots (Clontech, Palo Alto, CA; Biochain, Palo Alto, CA). Hybridization is conducted per the manufacturer's (Clontech) protocol using their Express Hyb hybridization solution. A glyceral-aldehyde-phosphate dehydrogenase (GAPDH) specific probe can be used to standardize for RNA loadings.

Northern blot analysis and cDNA library screens suggest that both IKR-1 and IKR-2 are widely expressed.

EXAMPLE 5: Measuring kinase activity of IKR-1 and IKR-2

Isolated IKR-1 or IKR-2 polypeptide or fusion proteins containing the isolated protein kinase domain of IKR-1 or IKR-2 can be used in an assay of protein kinase activity. Typically this would be carried out by combining IKR-1 or IKR-2 with radiolabeled ATP ($\gamma^{32}\text{P}$ -ATP) and a magnesium salt in buffer solution containing a peptide or protein substrate. The peptide substrates are generally from 8-30 amino acids in length and may terminate at the N- or C-terminus with three or more lysine or arginine residues to facilitate binding of the peptide to phosphocellulose paper. The substrate may also be a protein known to be phosphorylated readily by IKR-1 and/or IKR-2. Many such general kinase substrates are known, e.g., α or β casein, histone H1, myelin basic protein, etc. After incubation of this reaction mixture at 20-37°C for a suitable time, the transfer of radioactive phosphate from ATP to the substrate protein

or substrate peptide may be monitored, by spotting of the reaction mixture onto phosphocellulose paper, and subsequent washing of the paper with a dilute solution of phosphoric acid, in the case of a peptide substrate, or by application of the reaction products to a gel electrophoresis system followed by autoradiographic detection in the case of proteins. Other methods well known in the art are available to conveniently measure the IKR-1 and/or IKR-2-mediated transfer of phosphate to substrate proteins, such as the scintillation proximity assay.

The specification is most thoroughly understood in light of the teachings of the references cited within the specification which are hereby incorporated by reference. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention.

What is claimed is:

1. An isolated IKR-1 or IKR-2 nucleic acid selected from the group consisting of:
 - (a) the DNA sequence of SEQ ID NO:1 or SEQ ID NO:3;
 - (b) an isolated nucleic acid encoding an amino acid sequence comprising the sequence of SEQ ID NO:2 or SEQ ID NO:4;
 - (c) an isolated nucleic acid that hybridizes to either strand of a denatured DNA molecule comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency;
 - (d) an isolated nucleic acid derived by *in vitro* mutagenesis from SEQ ID NO:1 or SEQ ID NO:3;
 - (e) an isolated nucleic acid degenerate from SEQ ID NO:1 or SEQ ID NO:3 as a result of the genetic code; and
 - (f) an isolated nucleic acid selected from the group consisting of mouse IKR-1 or IKR-2 DNA, human IKR DNA, an allelic variant of mouse IKR-1 or IKR-2 DNA, an allelic variant of human IKR DNA, and a species homolog of IKR DNA.
2. A recombinant vector that directs the expression of the nucleic acid of claim 1.
3. An isolated polypeptide encoded by the nucleic acid of claim 1.
4. An isolated polypeptide according to claim 3 having a molecular weight of approximately 81 kD or 83 kD as determined by SDS-PAGE.

5. An isolated polypeptide according to claim 3 in non-glycosylated form.
6. Isolated antibodies that bind to a polypeptide of claim 3.
7. Isolated antibodies according to claim 6, wherein the antibodies are monoclonal antibodies.
8. A host cell transfected or transduced with the vector of claim 2.
9. A method for the production of IKR-1 or IKR-2 polypeptides comprising culturing a host cell of claim 2 in a suitable culture medium under conditions promoting gene expression, and recovering said the polypeptide from the culture medium.
10. The method of claim 9, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.
11. A method for determining the molecular weight of a sample protein comprising comparison of the molecular weight of a sample protein with the molecular weight of a polypeptide of claim 3, wherein said comparison comprises:
 - application of said sample protein and polypeptide to an acrylamide gel;
 - resolution of said sample protein and polypeptide using an electric current; and
 - application of a reagent for detecting said protein and polypeptide.

12. A kit for determining the molecular weights of peptide fragments of a sample protein comprising:

a vessel;

a polypeptide of claim 3;

at least one enzyme selected from the group consisting of

Asparaginylendopeptidase, Arginylendopeptidase, *Achromobacter* protease I, Trypsin, *Staphylococcus aureus* V8 protease, Endoproteinase Asp-N, and Endoproteinase Lys-C;

a mutant derived from said polypeptide by *in vitro* mutagenesis, wherein a site of cleavage by said enzyme on said polypeptide has been removed; and

fragmented peptides derived from said polypeptide by cleavage with said enzyme;

wherein said polypeptide and said sample protein are contacted with said enzyme, and said sample protein, polypeptide, and fragmented peptides are detected by a process comprising the steps of:

application of said protein, sample polypeptides, and fragmented peptides to an acrylamide gel;

resolution of said sample protein, polypeptide, and fragmented peptides using an electric current; and

application of a reagent for detecting said sample protein, polypeptides and said fragmented peptides.

13. An IKR-1 or IKR-2 polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) amino acids 1-300 of SEQ ID NO:2; and

(b) amino acids 1-300 of SEQ ID NO:4.

14. A method of determining protein kinase activity in which the protein kinase moiety is the IKR-1 or IKR-2 polypeptide of claim 3, 4, 5, or 13.

15. A method for identifying substances that affect phosphorylation comprising:

- (a) providing a substance suspected of affecting phosphorylation activity;
- (b) adding, in any order
 - (1) a peptide or protein substrate to be phosphorylated; and
 - (2) a polypeptide having kinase activity selected from the group consisting of IKR-1 (SEQ ID No. 2), IKR-2 (SEQ ID No. 4), an active domain thereof, and a fragment thereof;
 - (3) a source of phosphates;
- (c) incubating for a time and under conditions sufficient for kinase-mediated transfer of a phosphate to the substrate;
- (d) measuring the amount of phosphate transferred;
- (e) comparing the amount of phosphate transferred to that transferred in the presence of a standard substance of known phosphorylation-affecting activity

16. The method of claim 15 wherein the substrate interferes with kinase-mediated phosphorylation.

17. The method of claim 15 wherein the substrate enhances kinase-mediated

phosphorylation.

18. A method of identifying substances that interfere with the activation of NF- κ B comprising:

- (a) providing a substance suspected of interfering with the activation of NF- κ B;
- (b) adding, in any order:
 - (1) peptides or protein substrates to be phosphorylated;
 - (2) a polypeptide having kinase activity selected from the group consisting of IKR-1 (SEQ ID NO:2), IKR-2 (SEQ ID NO:4), an active domain thereof, and a fragment thereof;
 - (3) adenosine triphosphate (ATP);
 - (4) a gene with an intact promoter that is activated by NF- κ B; and
 - (5) all other factors necessary for transcription;
- (c) incubating the components for a time and under conditions sufficient for kinase function and transcription of the gene;
- (d) measuring the amount of transcript made; and
- (e) comparing the amount of transcript made to a standard to determine if the substance interferes with NF- κ B activation.

19. The isolated nucleic acid of claim 1, wherein said conditions of moderate stringency comprise hybridization in 50% formamide and 6x SSC at 42°, and washing in 0.5x SSC and 0.1% SDS at 60°C.

20. The polypeptide of claim 3, wherein said polypeptide is a protein kinase.

21. A method of inhibiting the kinase activity of IKR-1 or IKR-2 in a mammal, said method comprising administering to said mammal an effective amount of a compound that inhibits the kinase activity of a polypeptide comprising the sequence of SEQ ID NO:2 or a fragment thereof or SEQ ID NO:4 or a fragment thereof.

22. A method of enhancing the kinase activity of IKR-1 or IKR-2 in a mammal, said method comprising administering to said mammal an effective amount of a compound that enhances the kinase activity of a polypeptide comprising the sequence of SEQ ID NO:2 or a fragment thereof or SEQ ID NO:4 or a fragment thereof.

23. A method of treating an animal having a disease characterized by an overproduction or upregulated production of IKR-1 or IKR-2, said method comprising administering to said mammal a composition comprising an effective amount of a compound that inhibits the kinase activity of a polypeptide comprising the sequence of SEQ ID NO:2 or a fragment thereof or SEQ ID NO:4 or a fragment thereof.

24. A method of treating an animal having a disease characterized by an underproduction or downregulated production of IKR-1 or IKR-2, said method comprising administering to said mammal a composition comprising an effective amount of a compound that enhances the kinase activity of a polypeptide comprising the sequence of SEQ ID NO:2 or a fragment thereof or SEQ ID NO:4 or a fragment thereof.

25. A method of designing a molecule that inhibits the kinase activity of a polypeptide according to claim 20, said method comprising the steps of:

- determining the three dimensional structure of said polypeptide;
- analyzing said three dimensional structure for the likely binding sites of substrates;
- synthesizing a molecule that incorporates a predictive reactive site; and
- determining the kinase-inhibiting activity of the molecule.

26. A method of designing a molecule that increases the kinase activity of a polypeptide according to claim 20, said method comprising the steps of:

- determining the three dimensional structure of said polypeptide;
- analyzing said three dimensional structure for the likely binding sites of substrates;
- synthesizing a molecule that incorporates a predictive reactive site; and
- determining the kinase-enhancing activity of the molecule

NAME:IKR-1

Nucleotide sequence:

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1   CACAGGAAAC AGCTATGACC ATGATTACGC CAAGCTCGAA ATTAACCCTC
51  ACTAAAGGGA ACAAAGCTG GAGCTCCACC GCGGTGGCGG CCGCTCTAGA
101 ACTAGTGGAT CCCCCGGGCT GCAGGAATTC CGGCCTGGGA CTGGGTACCC
151 CACTGCTCTC AGAGAGGCAG GAAAGAGACC TTCAGGCTCA AGACCATCAC
201 CTGCTTTGCC TGTGGATCCT GGGGGGCCCC ATAGCTACCA GGATCTTCTA
251 GGCAGTCCCC AGGATTGACT TCAAGGCTTG AATCCCTGGG GGTGCCACCC
301 AGTTCCACAA GTCTGCATTG CCCTGCAACT GAGATAGGAG ATGGGGAAGA
351 AGATAGCCAA GCCCAGGAGA TGCAGAGTAC CACTAACTAC CTGTGGCATA
401 CTGATGACCT GCTAGGGCAG GGGGCCACTG CCAGTGTGTA CAAGGCCCGA
451 AACAAAGAAAT CCGGGGAGGT GGTGCTGTA AAGGTCTTCA ACTCAGCCAG
501 CTATCGGCGA CCTCCTGAGG TTCAGGTGAG GGAGTTTGAG GTCCTGCGGA
551 GGCTGAATCA CCAGAACATC GTGAAGCTAT TCGCAGTGGA GGAAACGGGA
601 GGCAGCCGGC AGAAGGTGCT AATCATGGAG TACTGCTCCA GTGGGAGCCT
651 GCTGAGCGTG CTGGAAGACC CTGAGAACAC GTTCGGGCTT TCTGAAGAGG
701 AGTTCCTAGT GGTGCTGCGC TGTGTGGTGG CTGGCATGAA CCACCTGCGG
751 GAGAATGGCA TTGTCCATCG GGACATCAAA CCTGGGAACA TCATGCGCCT
801 GGTGGGCGAG GAGGGGCAGA GCATCTATAA GCTGTCTGAC TTCGGGGCTG
851 CCCGCAAGCT GGACGATGAT GAGAAGTTTG TTTCTGTCTA TGGTACAGAG
901 GAATACCTGC ACCCTGACAT GTATGAGCGT GCAGTGCTGC GCAAACCCCA
951 GCAAAGGCA TTTGGTGTGA CTGTGGATCT CTGGAGTATT GGGGTGACCC
1001 TGTACCACGC AGCCACAGGC AGTCTGCCCT TCATCCCCTT CGGTGGGCCC
1051 CGGCGCAACA AAGAGATCAT GTACAGAATC ACCACAGAGA AGCCAGCCGG
1101 GGCCATTTCA GGGACTCAGA AGCACGAAAA TGGTCCCTTG GAGTGGAGCT
1151 ACAGCCTCCC CATCACCTGT AGACTGTCCA TGGGACTGCA GAACCAGCTG
1201 GTGCCCATCC TGGCCAACAT CCTGGAGGTG GAAGAGGATA AGTGCTGGGG
1251 CTTTGATCAG TTCTTCGCGG AGACCAGTGA CATTCTGCAG CGAACGGTCA
1301 TCCACGTCTT TTCCCTACCC CAGGCCGTTT TGCATCATGT CTACATCCAC
1351 GCCACAACA CGATTGCCAT CTTTTTGGAG GCTGTATATG AGCAGACCAA
1401 CGTGACCCCC AAACACCAGG AGTACCTCTT CGAGGGTCAC CCTTGTGTCC
1451 TTGAGCCAAG CCTCTCAGCC CAGCACATCG CCCACACAGC TGCCAGCAGC
1501 CCTCTAACTC TGTTTCAGCAT GTCCAGCGAC ACACCTAAGG GGCTGGCCTT
1551 CAGGGACCCT GCTCTGGATG TCCCAAAGTT CGTCCCTAAG GTTGACCTAC
1601 AGGCCGATTA CAGCACAGCT AAGGGGGTGC TGGGCGCTGG CTACCAGGCC
1651 CTGTGGCTGG CGCGGGTCCT GCTGGATGGA CAGGCGTTGA TGCTTCGGGG
1701 GTTACATTGG GTCCTGGAGG TGCTTCAGGA CACGTGCCAG CAGACACTGG
1751 AGGTCACACG GACAGCCCTC CTCTACCTCA GCAGCAGCCT GGGCACTGAA
1801 AGGTTTCAGCA GTGGAGCGGG GATGCCTGAC GTCCAGGAAC GAAAGGAGGC
1851 CACAGAGCTA AGAACCAGGC TGCAGACTCT CTCAGAGATC CTGTCTAAAT

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1901	GTTCCACAA	TGTCACAGAA	ACCCAAAGGA	GCCTGAGCTG	TCTGGGTGAA
1951	GAGCTTTTAA	AGAACCGGGA	CCAGATTCAT	GAGGATAACA	AAAGTATCCA
2001	GAAGATTCAG	TGTTGTTTGG	ACAAGATGCA	CTTCATCTAC	AAACAGTTCA
2051	AGAAATCCAG	GATGAGGCCA	GGGCTCAGCT	ACAATGAGGA	GCAGATCCAC
2101	AAGCTGGATA	AGGTAAATTT	CAGTCATCTA	GCCAAGAGGC	TGCTGCAGGT
2151	GTTCCAGGAG	GAGTGTGTGC	AGACGTATCA	GGTGTCGCTG	GTCACACACG
2201	GCAAGCGGAT	GAGGCAGGTG	CAGAGGGCCC	AGAACCACCT	GCATCTCATT
2251	GGCCACTCTG	TGGCCACCTG	TAACCTCGGA	GCCCGGGGAG	CCCAGGAGAG
2301	TCTGAACAAG	ATCTTTGATC	AGCTCCTTCT	GGACAGAGCT	TCCGAACAGG
2351	GAGCTGAGGT	GTCACCGCAA	CCTATGGCTC	CTCATCCCGG	CCCTGATCCG
2401	AAGGACCTGG	TCTTCCACAT	GCAGGAGCTT	TGTAATGATA	TGAAGCTATT
2451	GGCCTTTGAT	CTCCAGGACA	ACAACCGACT	CATCGAACGG	TTACATAGAG
2501	TTCCATCGGC	ACCAGATGTC	TGAGCTCCCT	GGGGGTTTAC	AAGGCACTCA
2551	GAAGCAATAG	AAACATTCAT	ATTGTACCCC	TACACTGTGA	GACCAAATTC
2601	AGGGCAAGTT	CTGGTTCCAT	CTCACTAGCC	TACCTCCCTC	TTGGCCATTG
2651	GCCATTGGCC	AACAAACTAG	CATTACTTTG	ACTGTCCTCT	TGGGAAGCAG
2701	CTAGGACAGG	GACTCCTGGC	CATCCCAGGC	AGTATCTACA	GAAGAGACCA
2751	TGCGGCTACC	ACAGCCTTAT	CAAGACACCA	AGACTGTTCT	TCCTCACCCA
2801	GGCTCTGGAG	GTCTGGTCTT	GGAAAGAAAA	GGCTCAGCCC	TCTCACGCTC
2851	TGCACTTCCC	AGGACCAGCA	GGCGTCTCCT	GTGGCTTCTC	CTGCCTCTCC
2901	AGGGTGCTGG	ATCAGAATGC	TTATTCTTGG	TTGTTTCTTG	TGCTGCTTCC
2951	TGAGTGTTCC	CATCCCTGGC	CTCAGGCAAC	CCACAAACGG	CCCCTCTGTG
3001	CTTGGTCTAG	ATGCACCTGC	ATTTGAGAAA	GTGGGTGGTT	GAGGCTAACT
3051	GCTGGTGCTT	TGAGGATTCT	CCTTGACCTT	TTCTCCGAGG	AACGCTTGGT
3101	TCTAAGAAAC	AGCTGGTCAG	TATCAACCAC	AGCCATGCTA	ACTGGACAGA
3151	TGTTGGAACC	CAAAGTCCTA	AGGAGAGAGC	AGGCCTGCAC	CTTCAGACAT
3201	GGAATAAATA	CATCGCCTTT	TCTGTTTAAA	AAAAAAAAAA	AAAAACCGGA
3251	ATTCGATATC	AAGCTTATCG	ATACCGTCGA	CCTCGAGGGG	GGGCCCCGGA
3301	CCCAATTTCG	CCTATAGTGA	GTCGTATTAC	AATTCACTGG	CCGTCGTTTT
3351	ACAACGTCGT	GACTGGGAAA	ACCCTGGCGT	TACCC	(SEQ ID NO:1)

Figure 1

NAME:IKR-2

Nucleotide sequence:

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1   TTGGGTAACG CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGCCA
51  GTGAATTGTA ATACGACTCA CTATAGGGCG AATTGGGTAC CGGGCCCCCC
101 CTCGAGGTCG ACGGTATCGA TAAGCTTGAT ATCGAATTCC GGCACGCGCG
151 GGCATACATG CAAATCTCTT CTTCCCCCTT ATCGTGAGGA GAAGCGCCTG
201 GACAAGCCGA GATGCAGAGC ACCTCCAACC ATCTGTGGCT CCTGTCTGAT
251 ATCCTAGGCC AGGGGGCCAC TGCAAATGTC TTCCGAGGAA GGCATAAGAA
301 AACTGGTGAT CTCTATGCTG TCAAAGTATT TAATAACATA AGCTTCCTTC
351 GCCCAGTGGA TGTTCAAATG AGAGAATTTG AAGTGTTAAA AAAACTCAAT
401 CACAAAAACA TTGTCAAGTT ATTTGCTATT GAAGAGGAGA CAACAACAAG
451 ACATAAAGTG CTTATTATGG AGTTTTGTCC CTGTGGGAGT TTATACTACTG
501 TTCTAGAGGA GCCGTCCAAT GCGTATGGAC TTCCAGAATC AGAATTTCTC
551 ATTGTCTTAC GAGATGTGGT GGGCGGGATG AATCATCTCC GAGAGAACGG
601 CATAGTGCAC CGAGATATCA AGCCAGGCAA CATCATGCGC GTCATAGGGG
651 AGGACGGCCA GTCTGTGTAC AAACCTCACGG ATTTCTGGCGC CGCTCGAGAG
701 CTGGAGGACG ATGAGCAGTT TGTGTCTCTG TACGGCACAG AAGAGTACCT
751 GCATCCGGAC ATGTATGAAA GGGCAGTGCT AAGAAAGGAC CATCAGAAGA
801 AGTACGGGGC TACCGTTGAT CTGTGGAGTG TTGGAGTGAC ATTCTACCAT
851 GCAGCCACGG GGTCGCTGCC GTTTAGACCC TTCGAGGGGC CTCGGAGGAA
901 CAAAGAAGTA ATGTATAAAA TAATCACTGG GAAGCCGTCT GGTGCAATAT
951 CTGGAGTACA GAAAGCAGAA AACGGACCAA TTGACTGGAG TGGAGACATG
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Figure 2

Name: IKR-1 (Polypeptide)

1	MQSTTNYLWH	TDDL LGQGAT	ASVYKARNKK	SGEVVAVKVF	NSASYRRPPE
51	VQVREFEVL	RLNHQNI	KL FAVEETG	GSR QKVLIME	YCS SGSLLSV
101	PENTFGLSE	E EFLVVL	R CVV AGMNHL	RENG IVHRDI	KPGN IMRLVGE
151	SIYKLSDFG	A ARKLDD	DEKF VSVYGTE	EYL HPDMYER	AVL RKPQQKA
201	TVDLWSIGV	T LYHAATG	SLP FIPFGG	PRRN KEIMYR	ITTE KPAGAI
251	KHENGPLEW	S YSLPITC	RLS MGLQNQ	L VPI LANILE	VEED KCWGF
301	ETSDILQRT	V IHVFSLP	QAV LHHVYI	HAHN TIAIFL	EAVY EQTNV
351	EYLFEGHPC	V LEPSLSA	QHI AHTAAS	SPLT LFSMSS	DTPK GLAFR
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451	VLQDTCQQT	L EVTRTAL	LYL SSSLGTE	RFS SGAGMP	DVQE RKEATE
501	LQTLSEILS	K CSHNVT	TETQR SLSC	LGEELL KNRD	QIHEDN KSIQ
551	DKMHFIYKQ	F KKSRRM	RPGLS YNEEQ	IIHKLD KVN	FSHLAKR LLQ
601	QTYQVSLV	T H GKRMRQ	VQRA QNHLH	LIGHS VATCN	SEARG AQESL
651	QLLLDRASE	Q GAEVSP	QPMA PHPGP	DPKDL VFHM	QELCND MKLL
701	NNRLIERL	H R VPSAPDV	(SEQ ID NO:2)		

Figure 3

Name:IKR-2 (Polypeptide)

1	MQSTSNHLWL	LSDILGQGAT	ANVFRGRHKK	TGDLYAVKVF	NNISFLRPVD
51	VQMREFEVLK	KLNHKNIVKL	FAIEEETTTR	HKVLIMEFCP	CGSLYTVLEE
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151	SVYKLTDFGA	ARELEDDEQF	VSLYGTEEYL	HPDMYERAVL	RKDHQKKYGA
201	TVDLWSVGVV	FYHAATGSLP	FRPFEGPRRN	KEVMYKIITG	KPSGAISGVQ
251	KAENGPIDWS	GDMPLSCSL	QGLQALLTPV	LANILEADQE	KCWGFDQFFA
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351	ELIYEGRRLV	LELGRLAQHF	PKTTEENPIF	VTSREQLNNT	GLRYEKISLP
401	KIHPRYDLVG	DASMAKAVTG	VVCYACRTAS	TLLLYQELMR	KGVRWLVELV
451	KDDYNETVHK	KTEVVITLDF	CIRNIEKTVK	VYEKLMKVNL	EAAELGEISD
501	IHTKLLRLSS	SQGTIESSLQ	DISSRLSPGG	LLADTWAHQE	GTHPRDRNVE
551	KLQVLLNCIT	EIYYQFKKDK	AERRLAYNEE	QIHKFDKQKL	YYHATKAMSH
601	FSEECVRKYE	AFKDKSEEW	RKMLHLRKQL	LSLTNQCFDI	EEEVSKYQDY
651	TNELQETLPQ	KMLAASGGVK	HAMAPIYPSS	NTLVEMTLGM	KKLKEEMEGV
701	VKELAENNIH	LERFGSLTMD	GGLRNVDC	(SEQ ID NO: 4)	

Figure 4

SEQUENCE LISTING

<110> Bird, Timothy A.
Virca, G.D.
Immunex Corp.

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INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/US 99/17578

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/55 C12N9/12 C07K16/40 G01N33/50 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NAGASE T. ET AL.: "PREDICTION OF THE CODING SEQUENCES OF UNIDENTIFIED HUMAN GENES. IV. THE CODING SEQUENCES OF 40 NEW GENES (KIAA0121-KIAA0160) DEDUCED BY ANALYSIS OF CDNA CLONES FROM HUMAN CELL LINE KG-1"</p> <p>DNA RESEARCH, vol. 2, no. 4, 1995, pages 167-174, XP000676653 ISSN: 1340-2838 cited in the application the whole document</p> <p style="text-align: center;">— — — — — -/-</p>	1-20, 25, 26

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"B" document member of the same patent family

Date of the actual completion of the international search

20 December 1999

Date of mailing of the international search report

11/01/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

Int. Patent Application No.

PCT/US 99/17578

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARRA M. ET AL. : "The WashU-HHMI Mouse EST Project." EMBL DATABASE ENTRY AA867810; ACCESSION NUMBER AA867810, 17 March 1998 (1998-03-17), XP002125811 abstract	1,19
X	US 5 776 717 A (CAO ZHAODAN) 7 July 1998 (1998-07-07) the whole document	1-11, 13-20, 25,26
X	MARRA M. ET AL.: "The WashU-HHMI Mouse EST Project." EMBL DATABASE ENTRY MMAA65250; ACCESSION NUMBER AA265250, 21 March 1997 (1997-03-21), XP002125812 abstract	1,19
X	MARRA M. ET AL.: "The WashU-HHMI Mouse EST Project." EMBL DATABASE ENTRY MMA59563; ACCESSION NUMBER AA059563, 24 September 1996 (1996-09-24), XP002125813 abstract	1,19
X	MARRA M. ET AL.: "The WashU-HHMI Mouse EST Project." EMBL DATABASE ENTRY MM1266689; ACCESSION NUMBER AA475369, 22 June 1997 (1997-06-22), XP002125814 abstract	1,19
P,X	MARRA M. ET AL.: "The WashU-NCI Mouse EST Project 1999." EMBL DATABASE ENTRY AI663156; ACCESSION NUMBER AI663156, 11 May 1999 (1999-05-11), XP002125815 abstract	1,19
P,X	WISNIEWSKI D. AND MARCY A. I.: "Mus musculus homolog of human T2K cDNA." EMBL DATABASE ENTRY AF145705; ACCESSION NUMBER AF145705, 3 June 1999 (1999-06-03), XP002125816 abstract	1-5, 8-10,13, 19
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INTERNATIONAL SEARCH REPORT

Int'l. Patent Application No.

PCT/US 99/17578

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>POMERANTZ J.L. AND BALTIMORE D.: "NF-(kappa)B activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase." THE EMBO JOURNAL, vol. 18, no. 23, 1 December 1999 (1999-12-01), pages 6694-6704, XP002125817</p>	<p>1-20, 25, 26</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/17578

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 21-24
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see ADDITIONAL sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26 (all partially)

IKR-1 and the nucleic acid encoding it; nucleic acid molecules hybridizing with said nucleic acid; a recombinant vector containing said nucleic acid; a host cell containing said vector; a method for the production of IKR-1; antibodies specific for IKR-1; a method for determining the molecular weight of a sample protein or peptide fragments; a method of determining protein kinase activity; a method for identifying substances that affect phosphorylation; a method of identifying substances that interfere with NF-(kappa)B; methods of inhibiting or enhancing IKR-1 activity in a mammal; methods of treating an animal having a disease characterized by over- or underproduction of IKR-1; and methods of designing molecules that inhibit or increase the kinase activity of IKR-1.

2. Claims: 1-26 (all partially)

IKR-2 and the nucleic acid encoding it; nucleic acid molecules hybridizing with said nucleic acid; a recombinant vector containing said nucleic acid; a host cell containing said vector; a method for the production of IKR-2; antibodies specific for IKR-2; a method for determining the molecular weight of a sample protein or peptide fragments; a method of determining protein kinase activity; a method for identifying substances that affect phosphorylation; a method of identifying substances that interfere with NF-(kappa)B; methods of inhibiting or enhancing IKR-2 activity in a mammal; methods of treating an animal having a disease characterized by over- or underproduction of IKR-2; and methods of designing molecules that inhibit or increase the kinase activity of IKR-2.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 21-24

Claims 21-24 refer to antagonists or agonists of IKR-1 or IKR-2 activity without giving a true technical characterization. Moreover, no such specific compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/17578

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5776717 A	07-07-1998	US 5837514 A	17-11-1998
		AU 6691398 A	22-09-1998
		WO 9839410 A	11-09-1998

Form PCT/ISA/210 (patent family annex) (July 1992)

